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Antimicrobial Susceptibility Testing in Host-Mimicking Media versus Standard Testing Media

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by

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ABSTRACT

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Antibiotic resistance has emerged as a serious threat to society and is responsible for at least 2 million illnesses each year. A common contribution to the spread of antibiotic resistance is the incorrect use of antibiotics. Prescription of the correct drug increases the chance of completely eliminating the infection early on and lowers the risk of selecting for resistant strains. Antimicrobial susceptibility testing (AST) results guide physicians in their decision of which antibiotic to prescribe. Current AST is carried out in a standardized manner *in vitro* which unfortunately does not consistently represent the outcome in the host environment. Improvements in AST methods must be implemented in order to provide a more accurate prediction of treatment outcome. We have introduced a novel approach to performing AST by substituting the standard media with host-mimicking media. We found in several cases that when host-mimicking media is used for testing, the susceptibility or resistance of a strain to a drug can be significantly altered and even change the prediction of whether drug will succeed or fail in the host. We also observed treatment outcomes *in vivo* that were consistent with the susceptibility profiles obtained with host-mimicking media but inconsistent with the susceptibility profiles obtained with standard media. Further, the addition of sodium bicarbonate ($NaHCO_3$) to standard testing medium often resulted in the same susceptibility

profiles that were obtained with host-mimicking media and linked to *in vivo* treatment outcomes. This indicates that the presence of certain signaling molecules in testing media could be a simple change that improves the accuracy of AST. Lastly, we investigated the role of fetal bovine serum (FBS) as an extracellular signal that contributed to changes in susceptibility profiles. Overall, these findings emphasize the importance of recapitulating the host environment to obtain more accurate AST results, correct prescription of drugs, and in turn reduced cases of antibiotic resistance.

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ABBREVIATIONS

AST	Antimicrobial Susceptibility Testing
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase-negative Staphylococci
DMEM	Dulbecco's Modified Eagle Media
FBS	Fetal Bovine Serum
I	Intermediate Breakpoint
MHB	Mueller-Hinton Broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant Staphylococcus aureus
MSSA	Methicillin-sensitive Staphylococcus aureus
$NaHCO_3$	Sodium bicarbonate
R	Resistant Breakpoint
S	Susceptible Breakpoint
WHO	World Health Organization

Chapter 1: Introduction

1.1 The Rise of Antibiotic Resistance

Long before the existence of modern antibiotics and the impact of human industrialization on the environment, microorganisms developed abilities to biosynthesize chemicals that were toxic to other bacteria, thus enabling their survival in the highly diverse and competitive natural environments such as soil and water (Finley et al., 2013). Along with these capabilities and the selective pressure to withstand these toxins in the microbial environment came the evolution of modes of resistance, by genetic mutation or acquisition of new DNA, to provide a competitive advantage for resistant bacteria (Allen et al., 2010; McManus, 1997).

Billions of years later, beginning with the discovery of penicillin by Alexander Fleming in 1928, modern medicine was transformed and humans entered the modern era of antibiotics (Sengupta, Chattopadhyay, & Grossart, 2013). However, the success in controlling bacterial infections with newly developed antibiotics was short-lived and the emergence of resistant strains continued. In the present day, the antibiotic resistance crisis is growing and is a result of the overuse and incorrect prescription of drugs combined with the lack of development of new antibiotics (Ventola, 2015). In fact, there is a direct relationship between antibiotic consumption and the emergence of resistant bacteria strains ("The antibiotic alarm," 2013). This is because treatment with antibiotics speeds up the selection for resistant bacteria by removing drug-sensitive bacteria and leaving behind resistant bacteria. Additionally, treating bacterial infections with the wrong antibiotic exposes the strain to sub-inhibitory antibiotic doses. Not only can this cause changes in gene

expression that impact virulence, but it also promotes antibiotic resistance via increased horizontal gene transfer and mutagenesis (Sengupta et al., 2013; Viswanathan, 2014). It is crucial to understand the mechanisms by which bacteria can become resistant so that more prudent decisions can be made regarding the prescription of antibiotics.

1.2 Mechanisms of Antibiotic Resistance

Antibiotic resistance can be acquired by mutation of existing DNA or by uptake of foreign DNA. In addition to spontaneous mutations that cause resistance, many of the genes that are known to enable antibiotic resistance are found on transposons, integrons, or plasmids which can be transferred to other bacteria (Allen et al., 2010). The most common way that bacteria acquire antibiotic resistance is horizontal gene transfer, which results in the movement of resistance genes between bacterial species as well as the acquiring of resistance mechanisms in bacteria that were previously harmless (Davies & Davies, 2010; Finley et al., 2013). The ability to acquire DNA molecules by natural transformation is conserved among a wide range of bacteria, indicating that this genetic trait is functionally important for survival in the environment (Thomas & Nielsen, 2005).

There are several mechanisms by which the transfer of genetic material enables bacteria to become resistant to an antibiotic. The bacteria may obtain genes encoding enzymes that destroy the antibacterial agent, such as beta-lactamase enzymes which provide multi-resistance to beta-lactam antibiotics like penicillins and Cephalosporins (McManus, 1997; Tenover, 2006). Additionally, bacteria may acquire efflux pumps that force the antibiotic out of the cell (Tenover,

2006). Bacteria may also obtain several genes that each play a role in a metabolic pathway to alter the bacterial cell wall and prevent the antimicrobial agent from binding. Further, mutations that downregulate porin genes limit the access of antibiotics into the cell. In each of these cases the bacteria prevent the antimicrobial agent from reaching its target and exerting its effect.

There are also multiple mechanisms by which spontaneous mutations in bacteria can lead to antibiotic resistance. First, these mutations can modify or eliminate the binding site of the target protein such that the drug can no longer bind(Tenover, 2006). Second, a mutation can either upregulate pumps that expel the drug from the cell or downregulate an outer membrane protein channel that is required for the drug to enter the cell. Third, a mutation can upregulate the production of enzymes that inactivate the antimicrobial agent. Similar to the transfer of genetic material, the mechanisms of spontaneous mutations enable antibiotic resistance by preventing the drug from reaching its target.

No matter the mechanism, the use of antibiotics selects for antibiotic resistance by killing the susceptible strains and allowing the newly resistant strains to survive and reproduce. To gain better control of the rapid spread of antibiotic resistance, it is necessary to implement effective screening methods that will correctly determine the optimal antibiotic for treatment. By prescribing the correct compounds we reduce instances of treatment failure and in turn prevent the selection and spread of resistant bacteria.

1.3 Antimicrobial Susceptibility Testing and Clinical Breakpoints

Antimicrobial susceptibility testing (AST) is a standardized method performed *in vitro* to determine the most effective antibiotic(s) to prescribe for a bacterial infection. Due to the many variables that affect AST protocols, the World Health Organization (WHO) standardized these methods across the world in 1950 (Wheat, 2001). The Clinical and Laboratory Standards Institute (CLSI) in the United States then developed AST guidelines which cover specifications regarding the testing media, incubation conditions, inoculum density, and antibiotic dilution ranges so that AST can be performed in a standardized manner across laboratory settings (Clinical and Laboratory Standards Institute, 2012a, 2014).

The *in vitro* AST methods begin with a bacterial isolate that is grown in the standard testing medium, Mueller Hinton Broth (MHB), then diluted to a specific cell density and exposed to a range of 2-fold serial dilutions of a particular drug. The "Minimum Inhibitory Concentration" (MIC) is the lowest concentration of drug at which no cell growth is observed after the incubation period. These results are then interpreted using clinical breakpoints that have been established by various organizations and the MIC values are categorized as "Susceptible", "Intermediate", or "Resistant" (Turnidge & Paterson, 2007). A "Susceptible" MIC implies that the bacterial isolate will be inhibited by a recommended dosage of an antimicrobial agent. An "Intermediate" MIC indicates that the antibiotic may or may not work and should be prescribed with caution and only when there are no other available therapeutic options. A "Resistant" MIC implies that the antimicrobial agent will likely fail *in vivo*. The purpose of clinical breakpoints is to enable clinical microbiology

laboratories to communicate AST results to the prescribers in a way that will optimize antibiotic selection for the treatment of a bacterial infection. However, the establishment and use of clinical breakpoints is under the assumption that the *in vitro* MIC is representative of *in vivo* susceptibility.

Although the standardization of AST is cost effective and allows for reproducibility of results across laboratories, there are underlying flaws to these methods that can ultimately result in the incorrect prescription of antibiotics. The misuse of antibiotics not only contributes to antibiotic resistance through the selection of resistant strains, but it can also result in the spread of infection and loss of a patient's life. Therefore, it is absolutely necessary to consider modifications to the traditional AST that may improve the accuracy of test results and provide a higher rate of treatment success.

1.4 Using Host-Mimicking Media for *in vitro* Testing

Bacteria utilize environmental signals to alter gene expression and adapt to their changing environments. One mechanism for this is to use a two-component regulatory system (TCRS), composed of an extracellular sensor and an intracellular transcription factor (Gunn, 2008; Mekalanos, 1992). In the presence of a particular environmental cue, the extracellular sensor turns "on" and activates the intracellular transcription factor typically by phosphorylation. This active transcription factor then activates or represses the expression of specific genes. PhoPQ is an example of a TCRS in *Salmonella* that is activated by low magnesium and mildly acidic pH in the environment and in turn induces changes in gene expression that enable survival within host macrophages (Groisman, 2001; Gunn & Richards, 2007; Miller, Kukral,

& Mekalanos, 1989). Additionally, there are many other environmental cues – temperature, CO_2, iron, and other compounds – that regulate virulence factors by playing a role in the highly complex signal transduction systems that bacteria have developed. Further, IVET (in vivo expression technology) was developed to identify genes expressed during infection (Mahan, Slauch, & Mekalanos, 1993). This system uses plasmid libraries containing a functional *in vivo* activated promotor fused to a gene required for growth *in vivo*, such that only strains that have taken up this plasmid will grow in the host. Identification of the promoters activated *in vivo* enables us to determine the downstream genes regulated by these promoters. This information proves useful in creating a media that will mimic the *in vivo* environment by activating virulence factors that are expressed *in vivo.* For example, a pH 5.5 media low in phosphate and magnesium was shown to activate the PhoPQ gene regulator and, as described previously (Gunn, 2008; Mekalanos, 1992), stimulate expression of genes necessary for growth within the host cells. Thus, it is clear that bacteria differentially express virulence genes depending on the media they are grown in.

While the established AST methods performed *in vitro* are often valuable and lead to correct antibiotic prescription and successful treatment of bacterial infections, this is not always the case. Gene expression in bacteria is highly regulated by the extracellular environment such that a strain may have altered susceptibility to a particular drug when grown in a different media. Therefore, it is incorrect to assume that the rich and universal testing media, MHB, closely represents the host environment and will provide accurate MIC values in every

case. This disconnect between *in vitro* testing and the true *in vivo* susceptibility leads to treatment with antibiotics that will not clear the infection as well as the ruling out of antibiotics that are quite capable of clearing the infection. By performing AST with strains grown in host-mimicking media, we can create an environment that better represents the body. We hypothesize that bacteria grown in host-mimicking media, specifically those grown in serum-like media, will demonstrate altered susceptibility to antibiotics compared to those grown in the standard MHB.

Chapter 2: Correcting a Fundamental Flaw in the Paradigm for Antimicrobial Susceptibility Testing[†]

[†]This chapter contains excerpts, reproduced with permission, from Ersoy SC *et al.* (2017) Correcting a Fundamental Flaw in the Paradigm for Antimicrobial Susceptibility Testing. *EBioMedicine.* (20): 173-181.

Summary of Contributions to Work

Assisted in the screening process to determine Minimum Inhibitory Concentration (MIC) of antibiotics for strains grown in host-mimicking media. Contributed significantly to MIC testing for all antibiotics and strains in media with and without sodium bicarbonate.

Abstract

The emergence and prevalence of antibiotic-resistant bacteria are an increasing cause of death worldwide, resulting in a global ‘call to action’ to avoid receding into an era lacking effective antibiotics. Despite the urgency, the healthcare industry still relies on a single in vitro bioassay to determine antibiotic efficacy. This assay fails to incorporate environmental factors normally present during host-pathogen interactions in vivo that significantly impact antibiotic efficacy. Here we report that standard antimicrobial susceptibility testing (AST) failed to detect antibiotics that are in fact effective in vivo; and frequently identified antibiotics that were instead ineffective as further confirmed in mouse models of infection and sepsis. Notably, AST performed in media mimicking host environments succeeded in identifying specific antibiotics that were effective in bacterial clearance and host survival, even

though these same antibiotics failed in results using standard test media. Similarly, our revised media further identified antibiotics that were ineffective in vivo despite passing the AST standard for clinical use. Supplementation of AST medium with sodium bicarbonate, an abundant in vivo molecule that stimulates global changes in bacterial structure and gene expression, was found to be an important factor improving the predictive value of AST in the assignment of appropriate therapy. These findings have the potential to improve the means by which antibiotics are developed, tested, and prescribed.

2.1 Introduction

Multidrug-resistant bacteria are a leading cause of death worldwide and undermine advances in medical and surgical management of multiple diseases (Centers for Disease Control and Prevention, 2013; World Health Organization, 2014). Despite this urgent threat (U.S. White House, 2015; World Health Assembly, 2014), the healthcare industry continues to rely on a single bioassay standardized in 1961 by the World Health Organization to determine antibiotic efficacy (World Health Organization, 1961). Although this bioassay has been immensely valuable for several decades, it is fundamentally flawed because it is based largely on in vitro efficacy, and often fails to correlate with patient outcome (Kubicek-Sutherland et al., 2015). Reliance on this bioassay may have inadvertently contributed to the rise in multidrug-resistant bacteria because it disqualifies efficacious compounds (Diene & Rolain, 2014).

A key parameter that guides decisions regarding antimicrobial therapy is the clinical breakpoint: the antimicrobial concentrations that are used to define isolates

as susceptible (“S”), intermediate (“I”), or resistant (“R”) (Clinical and Laboratory Standards Institute, 2012a; European Committee on Antibiotic Susceptibility Testing, 2014). Clinical breakpoints are established by a sequential procedure. (1) In vitro efficacy is assessed by standard antimicrobial susceptibility testing (AST), which determines the minimum inhibitory concentration (“MIC”) of antibiotics to which a pathogen is sensitive. (2) Pharmacokinetic/pharmacodynamic (PK/PD) parameters are measured in animals (dosing, distribution, localization). (3) Efficacy/toxicity is established in animals for a limited number of model pathogens. (4) Dosing protocols are validated with limited patient clinical data. Unfortunately, this testing pipeline is fundamentally unsound because the first step, AST, is performed on Mueller-Hinton Broth (MHB), a rich laboratory medium that fails to recapitulate most aspects of host environments. So, the fact that clinical breakpoints are based on a foundational assay performed in vitro raises questions as to how relevant they are to patient outcome.

Supporting this notion, several reports suggest that the clinical predictive value of AST in the assignment of appropriate therapy is limited. (1) Clinical observations have given rise to the “90–60” rule: “susceptible” infections respond well to appropriate therapy in 90% of cases, whereas “resistant” infections respond well to these antibiotics in 60% of cases (Doern & Brecher, 2011; Rex & Pfaller, 2002). (2) Pneumococcal patients treated with antibiotics that failed standard tests (discordant therapy) had similar treatment outcomes as those that passed standard tests (concordant therapy) (Yu et al., 2003). (3) AST-recommended antibiotics failed to clear *Salmonella enterica* Typhimurium and *Enterobacter cloacae* in murine models

of sepsis (Band et al., 2016; Kubicek-Sutherland et al., 2015). (4) An AST-disqualified antibiotic cleared multidrug-resistant Gram-negative pathogens in murine pulmonary models of infection (Lin et al., 2015). Here we propose that the antimicrobial testing assay should be revamped to account for pathogen conditions in the host, and show several circumstances in which susceptibility testing in host-mimicking media is more accurate than standard AST in predicting antibiotic efficacy in vivo. We have termed this behavior in vivo altered susceptibility (IVAS), providing insight into why some patients fail to respond to certain antibiotics despite passing standard tests for clinical use.

2.2 Results

2.2.1 Antibiotic MICs Are Markedly Different When Derived From Host-mimicking Media vs. Standard MHB Medium

A collection of human and veterinary clinical isolates was subjected to antimicrobial susceptibility testing in host-mimicking media vs. standard MHB medium. Four host-mimicking media were examined including (i) Dulbecco's Modified Eagle Medium (DMEM), a tissue culture medium supporting mammalian cell growth (Dulbecco & Freeman, 1959); (ii) Lacks medium, supporting pneumococcal growth (Lacks, 1966; Trombe, Clavé, & Manias, 1992); (iii) modified Lacks medium (MLM), simulating the nasopharynx for invasive pneumococcal carriage (Hathaway et al., 2012); and (iv) low-phosphate, low-magnesium medium (LPM pH 5.5), simulating the macrophage phagosome in which many intracellular pathogens reside/replicate (Coombes, Brown, Valdez, Brumell, & Finlay, 2004; Steele-Mortimer, 2008). Emphasis was placed on the identification of pathogen-

antibiotic combinations that exhibited altered MICs from host-mimicking media relative to standard MHB medium; and whose MICs crossed clinical breakpoint designations that are used to define isolates as susceptible (“S”), intermediate (“I”), or resistant (“R”), and can impact clinical decision making on appropriate antibiotic therapy. Thus, we sought to identify antibiotics for which a given pathogen is classified as “S” in MHB medium but “R” in host-mimicking media (S to R); and antibiotics for which a given pathogen is classified as “R” in MHB medium but “S” in host-mimicking media (R to S).

Staphylococcus (MRSA; MSSA; CoNS)

A panel of antibiotics used in human and veterinary medicine was tested for efficacy against clinical isolates of methicillin-resistant and -sensitive *S. aureus* (MRSA/MSSA), and coagulase negative *Staphylococcus* (CoNS) (**Fig. 1**). Growth of *Staphylococcus* in tissue culture medium and modified Lacks medium conferred increased susceptibility to azithromycin, erythromycin, and streptomycin relative to MHB medium (4 to 256-fold; **Fig. 1a**). Conversely, *Staphylococcus* exhibited increased resistance to daptomycin and rifampin in modified Lacks medium, and to tetracycline in tissue culture medium, relative to MHB medium (4 to 16-fold). **Table 1** lists pathogen-antibiotic combinations that exhibited at least an 8-fold change in MIC when derived in host-mimicking media vs. standard MHB medium and whose altered MICs crossed clinical breakpoint designations that advise on patient therapy. For example, antibiotics for which MRSA was classified as “R” in MHB medium, but classified as “S” in tissue culture medium (cephalothin); and antibiotics for which MSSA was classified as “I” in MHB medium, but classified as “S” in tissue

culture medium (erythromycin) (**Table 2a**). Notably, although many pathogen-antibiotic combinations have significant changes in MIC in host-mimicking media, many do not cross breakpoint designations (R to S; S to R) and would not alter physician making on appropriate therapy. For example, 3/3 MRSA isolates exhibited a 4- to 32-fold increased susceptibility to oxacillin in tissue culture medium, but the "altered MICs" of two MRSA isolates did not cross clinical breakpoints. Thus, they remain "Resistant" to oxacillin as defined by AST standards for clinical use.

S. pneumoniae

Altered MICs were also examined for *S. pneumoniae* clinical isolates tested in host-mimicking media vs. standard MHB medium. Most *S. pneumoniae* strains tested showed increased susceptibility to azithromycin in tissue culture medium and modified Lacks medium relative to MHB medium; and increased resistance to daptomycin and trimethoprim in modified Lacks medium (4 to 32-fold; **Fig. 1b**). Many *S. pneumoniae* MICs derived in host-mimicking media crossed clinical breakpoint designations (listed in **Table 1**); e.g., antibiotics for which *S. pneumoniae* was classified as "S" in MHB medium, but classified as "R" in modified Lacks medium (trimethoprim); and those for which *S. pneumoniae* classified was "R" in MHB medium, but classified as "S" in modified Lacks medium (azithromycin) (**Table 2b**).

Gram-negative Bacteria

Antibiotic efficacy was also examined for Gram-negative bacterial isolates tested in host-mimicking media vs. standard MHB medium. A subset of these antibiotics

(10 of 20), which were not subject to acute pH and/or media composition effects under LPM pH 5.5 conditions (Kubicek-Sutherland et al., 2015), were also interrogated. Several Gram-negative bacteria were associated with increased resistance to colistin or polymyxin B in tissue culture medium and LPM pH 5.5 conditions relative to MHB medium (4 to 512-fold) (**Fig. 1c**). Growth of *Yersinia* spp. (4 of 4 isolates) was associated with increased susceptibility to trimethoprim and co-trimoxazole in tissue culture medium relative to MHB medium (8 to 64-fold). Many Gram-negative bacteria MICs derived in host-mimicking media crossed clinical breakpoint designations (listed in **Table 1**); e.g., *Salmonella* Typhimurium (*ST*) susceptibility to colistin was classified as "S" in MHB medium but "R" in tissue culture medium (**Table 2c–f**).

Comparison Summary of MICs Derived From Host-mimicking Media vs. Standard MHB Medium

We evaluated the percentage of pathogen-antibiotic combinations that resulted in altered MICs when derived from host-mimicking media vs. standard MHB medium (**Fig. 2a**). Although the MICs obtained from host-mimicking media were comparable to those from MHB medium for approximately two-thirds of cases tested (852/1311), one third of these cases exhibited at least a 4-fold change in MIC, which may signal altered antibiotic susceptibility in vivo. Further, 8.2% (107/1311) of altered MICs derived from the host-mimicking media tested resulted in a change in clinical breakpoint designation, which may impact physician decision making (**Fig. 2b**). Taken together, these data suggest that inclusion of environmental factors normally present during host-pathogen interactions may

improve the predictive value of standard AST in identifying effective antibiotics to treat microbial infections.

2.2.2 Drug Testing in Host-mimicking Media Improves the Assignment of Appropriate Antibiotic Therapy

Several pathogen-antibiotic combinations that exhibited altered MICs in host-mimicking media were tested for efficacy in murine models of sepsis. We focused on antibiotics whose MICs exhibited at least an 8-fold altered susceptibility in host-mimicking media relative to standard MHB medium, and whose MICs crossed clinical breakpoint designations. This analysis was limited to human and veterinary clinical isolates that also infect mice.

MRSA, MSSA

All mice (10/10) survived infection with MRSA (USA300) following treatment with cephalothin or ceftriaxone (**Fig. 3a** ; $P < 0.001$), identified as efficacious in tissue culture medium even though these agents failed standard testing in MHB medium (R to S; R to I; **Table 2a**). Similarly, nearly all mice (8/10) survived MSSA (MT3307) infection following treatment with erythromycin ($P < 0.001$), identified as bioactive in tissue culture medium but relatively ineffective by standard testing (I to S). Treatment with co-trimoxazole, often used clinically (Holland et al., 2014), failed to improve survivorship (1/10; $P = 1.0$), as predicted by testing in tissue culture medium but not MHB medium (S to R).

Further analysis was done using a MRSA isolate (MT3302) linked to a fatal case of human sepsis. AST in host-mimicking media was evaluated in an effort to

retroactively identify alternative therapeutic options. Treatment with cephalosporins (ceftriaxone or ceftiofur) resulted in high efficacy in murine models of MRSA sepsis (8/10; 7/10; $P < 0.001$; $P < 0.01$). Both of these antibiotics were identified as efficacious in tissue culture medium even though they were rejected by standard testing (R to I). Further, all mice (10/10) survived treatment with daptomycin and ciprofloxacin (**Fig. 3a**; $P < 0.001$), as predicted by testing of daptomycin in standard MHB medium and tissue culture medium; and of ciprofloxacin in all media examined (**Table 2a**). Notably, testing of daptomycin in modified Lacks medium predicted resistance (S to R), indicating that this drug may be effective against certain types of infections but not others (e.g., systemic vs. localized).

S. pneumoniae

Despite passing standard testing in MHB medium, trimethoprim failed to protect mice (0/10) from *SPN* infection (strain Daw 25) (**Fig. 3b**; $P = 1.0$), as predicted by testing in modified Lacks medium (S to R; **Table 2b**). Further, all mice (10/10) survived following treatment with ceftriaxone ($P < 0.001$), for which susceptibility was indicated in all media tested.

Gram-negative Bacteria

Colistin, a drug of last resort (Yahav, Farbman, Leibovici, & Paul, 2012), failed to protect mice (1/10) from infection with *S*. Typhimurium (*ST*14028) (**Fig. 3c**; $P = 1.0$), as predicted by testing in tissue culture medium (S to R; **Table 2c**). Conversely, all mice (10/10) survived treatment with ciprofloxacin ($P < 0.001$), for which susceptibility was indicated in all media tested. Additionally, most mice (8/10)

survived infection with *K. pneumoniae* following treatment with tetracycline ($P < 0.001$). Such efficacy was predicted by standard testing in MHB and LPM pH 5.5 media (S to S), which mimics the macrophage phagosome wherein *K. pneumoniae* resides and replicates during infection (Cano et al., 2015) (**Table 2f**). Such efficacy was comparable to treatment with ciprofloxacin (8/10; $P < 0.001$) that has established activity against intracellular pathogens (Carryn et al., 2003). Notably, testing of tetracycline in tissue culture medium predicted resistance (S to R), suggesting that testing in media that reflect the intracellular lifestyle of *K. pneumoniae* is a more accurate predictor of treatment outcome for this pathogen.

Bacterial Clearance

Bacterial clearance from circulation in the blood was investigated following treatment with antibiotics predicted as highly efficacious by testing in standard MHB medium (co-trimoxazole) or tissue culture medium (azithromycin), respectively (**Table 2a**). Treatment with the AST-recommended antibiotic, co-trimoxazole, was ineffective in MSSA (MT3307) clearance as predicted by testing in host-mimicking media (S to R) (**Fig. 3d**). This treated cohort exhibited a progressive bacteremia (up to 2.5 × 105 colony forming units (CFU)/ml blood by day 6), with all mice (10/10) succumbing to infection by day 10 (open boxes). Such efficacy was comparable to that of untreated animals (open circles). Conversely, as predicted by testing in tissue culture medium, azithromycin was able to clear MSSA from circulation, with all mice (10/10) surviving the infection and harboring ≤ 2 × 103 CFU/ml in the blood at day 10 (closed boxes; $P < 0.001$). These data suggest that drug testing in host-

mimicking media improves the predictive value of standard AST in the assignment of appropriate therapy.

2.2.3 Addition of $NaHCO_3$ to Standard MHB Medium Improves the Accuracy of Antibiotic Efficacy In Vivo

We suspected that sodium bicarbonate ($NaHCO_3$) may be a key in vivo molecule contributing to antibiotic susceptibility for a number of pathogens for the following reasons. $NaHCO_3$ serves as an abundant ionic factor present in mammalian tissues that stimulates global changes in bacterial structure, gene expression, and membrane permeability that correspond to increased susceptibility to human cationic antimicrobial peptides (Dorschner et al., 2006). $NaHCO_3$ is present in nearly all host-mimicking media examined that resulted in altered antibiotic susceptibility relative to MHB medium. Thus, we evaluated whether supplementation of standard MHB medium with physiological levels of $NaHCO_3$ improved the predictive value of the AST standard for clinical use. This analysis was initially focused on *Staphylococcus*-antibiotic combinations that exhibited at least an 8-fold change in MIC in tissue culture medium vs. MHB medium, representing 13.5% (31/230) of combinations examined (**Fig. 1a**, top panel).

We investigated the fold-change between MICs derived in MHB medium in the presence/absence of $NaHCO_3$ (test/standard condition; left of slash); and in tissue culture medium in the absence/presence of $NaHCO_3$ (test/standard condition; right of slash) (**Fig. 4a** ; **Table 3a**). Increased susceptibility is depicted in blue; increased resistance is depicted in red. Four phenotypic classes were identified.

Class 1 (21/31). Addition of $NaHCO_3$ to MHB medium resulted in MICs similar to tissue culture medium; its removal from tissue culture medium resulted in MICs similar to MHB medium (azithromycin, erythromycin, tetracycline).

Class 2 (5/31). Addition of $NaHCO_3$ to MHB medium resulted in MICs similar to tissue culture medium; its removal from tissue culture medium had no effect on the MIC (ceftriaxone, ceftiofur).

Class 3 (2/31). Addition of $NaHCO_3$ addition to MHB medium had no MIC effect; its removal from tissue culture medium resulted in MICs similar to MHB medium (oxacillin).

Class 4 (3/31). Addition/removal of $NaHCO_3$ had no effect on MICs in MHB medium or tissue culture medium (trimethoprim). These data indicate that addition of $NaHCO_3$ to MHB medium restored the altered susceptibility observed in tissue culture medium in 83.9% (26 of 31) of cases tested.

Next, we examined whether physiological levels of $NaHCO_3$ in MHB medium were required to stimulate the altered susceptibility observed in tissue culture medium. A dose response analysis of MRSA (USA300; MT3302) and MSSA (MT3307) strains revealed that physiological levels of $NaHCO_3$ (~25 mM) (Mayo Clinic, 2017) were necessary to induce altered antibiotic susceptibility in MHB medium (**Fig. 4b**). These data suggest that $NaHCO_3$ may be a key in vivo component contributing to antibiotic susceptibility for a number of pathogens. Supporting this suggestion, supplementation of MHB medium with physiological levels of $NaHCO_3$ also resulted in altered drug susceptibilities in *S. pneumoniae* and

Salmonella spp. isolates (**Fig. 4c**; **Table 3b, c**). Further, many altered MICs crossed clinical breakpoint designations (listed in **Table 1**), and such predicted changes in antibiotic efficacy were confirmed in mouse models of infection and sepsis (**Fig. 2a**); e.g., MRSA (cephalothin [R to S]; ceftriaxone [R to I]); and MSSA (erythromycin [I to S]); (**Table 3a**). These findings suggest that supplementation of standard MHB medium with physiological levels of $NaHCO_3$ improved the predictive value of AST in the assignment of appropriate antibiotics for therapeutic intervention.

2.3 Discussion

Multidrug-resistant bacteria are a significant cause of sepsis, the most common cause of death in hospitalized patients, with an annual incidence of 1 million cases and 200,000 deaths in the U.S. alone (Deutschman & Tracey, 2014). This dire perspective reflects the failed efforts to fully contain bacteria with the misuse of antibiotics, and the legal, financial, and scientific hurdles to discovering new ones. We demonstrate that one viable approach to address this alarming threat is to incorporate host-mimicking media in standard AST methods for clinical use. Validation of the improved predictive value of AST in the assignment of appropriate antibiotic therapy was provided in several Gram-positive and -negative animal models of infection and sepsis. Our findings suggest that standard AST may be hindering optimal patient treatment, and slowing the process of discovery of new, effective, and safe antibiotics because it disqualifies efficacious compounds. Susceptibility testing that accounts for the biology of a pathogen in the context of its host may enable the re-purposing of omitted antibiotics while aiding the discovery of

new ones by screening compounds under conditions that more accurately reflect the host milieu.

Altered drug susceptibility in vivo provides insight as to why some patients fail to respond to certain antibiotics despite passing standard susceptibility tests. Our findings with a MRSA isolate from a deceased patient provide a clear example as antibiotics omitted by standard AST were highly efficacious in bacterial clearance. If these alternative therapeutic options had been made available to clinicians managing this case, it may have changed the patient outcome. Additionally, we show that supplementation of standard MHB medium with physiological levels of sodium bicarbonate improved the predictive value of AST in the assignment of appropriate therapy. The molecular basis likely involves the role of $NaHCO_3$ as an abundant ionic factor that stimulates global changes in bacterial structure and gene expression, leading to alterations in bacterial cell wall thickness and membrane permeability that correspond with increased susceptibility to human cationic antimicrobial peptides (Dorschner et al., 2006). Two potential alternative mechanisms include the role of bicarbonate in the maintenance of blood pH (Hermansen & Osnes, 1972; Rosenthal, 1948); and/or the inhibition of growth and viability of periodontal pathogens (Newbrun, Hoover, & Ryder, 1984). However, these mechanisms are unlikely to play a role in the improved predictive value of AST due to the inclusion of Tris buffer in the test media to preclude bicarbonate-mediated pH fluctuations that can affect antibiotic potency and bacterial cell viability.

Standard AST in clinical use has likely contributed to the alarming rise of multidrug-resistant bacteria in hospitals because high doses of ineffective antibiotics

are given to infected patients without the knowledge that the host environment may render bacteria inherently resistant to the antibiotics prescribed to kill them. Based on the findings of this study, rather than extending the dose/duration of an antibiotic that is not effective, physicians might consider that the more appropriate approach is to prescribe a totally different antibiotic. Standard AST in combination with host-mimicking media may serve as a valuable tool in advising clinicians on appropriate antibiotic therapy. Antibiotics identified by both approaches were efficacious in every animal model examined; thus, such cases should bestow high confidence in clinical decision making on appropriate therapy. Conversely, physicians should exercise caution in cases where marked MIC disparities occur between testing in host-mimicking media vs. standard MHB medium. Further, predicted drug failure in a particular host-mimicking media may indicate that certain drugs may be effective against certain types of infections but not others (e.g., systemic vs. localized). Supporting this suggestion, MRSA inactivates daptomycin by releasing membrane phospholipids under certain experimental conditions (Pader et al., 2016); and herein we show that a MRSA isolate was susceptible to daptomycin in tissue culture medium and in a murine model of sepsis, but displayed resistance in other host-mimicking media examined (minimal Lacks medium).

Future considerations must be given to host-pathogen interactions that can also influence drug susceptibility. (1) Animals, including primates, often tolerate drugs differently than humans (pharmacokinetic parameters such as drug clearance, volume of distribution, and half-life can result in unanticipated changes in antimicrobial efficacy) (Ambrose et al., 2007; Deziel et al., 2005). (2) Bacterial

community composition can compromise antibiotic efficacy (antibiotic deactivation or biofilm production provides passive resistance for all microbes within a polymicrobial environment) (Sorg et al., 2016; Vega & Gore, 2014). (3) Antimicrobial selection is based on drug concentrations achieved in plasma, but concentrations achieved in different tissues and sites of infection may be greater or less depending on the drug's properties (pH at the infection site or within an organelle can dictate lipid solubility of the drug or its distribution in cells and tissues) (Logan, Funk, Axcell, & Krise, 2012). (4) Antibiotic resistance may be inadvertently triggered by diet, underlying conditions in the patient, or by clinical interventions that may disrupt drug efficacy (ascorbic acid treatment of urinary tract infections to lower urine pH) (Carlsson, Wiklund, Engstrand, Weitzberg, & Lundberg, 2001). (5) Many patients that develop multidrug-resistant infections have co-morbidities, immunosuppressive therapy and/or the presence of invasive medical devices that impact susceptibility to indicated pathogens (Paterson & Bonomo, 2005).

Our findings suggest that the susceptibility testing in media that reflect the host milieu will not only improve the predictive value of AST in the assignment of appropriate antibiotic therapy, but also provides a new paradigm for drug discovery and therapeutic intervention for infectious diseases. However, such testing will always be open to further improvement, especially as we learn more about the subtle nuances of host-pathogen interactions in natural environments that influence the impact of antibiotics on bacterial clearance (e.g., virulence factors, ecological factors, and cell physiological parameters).

Figures

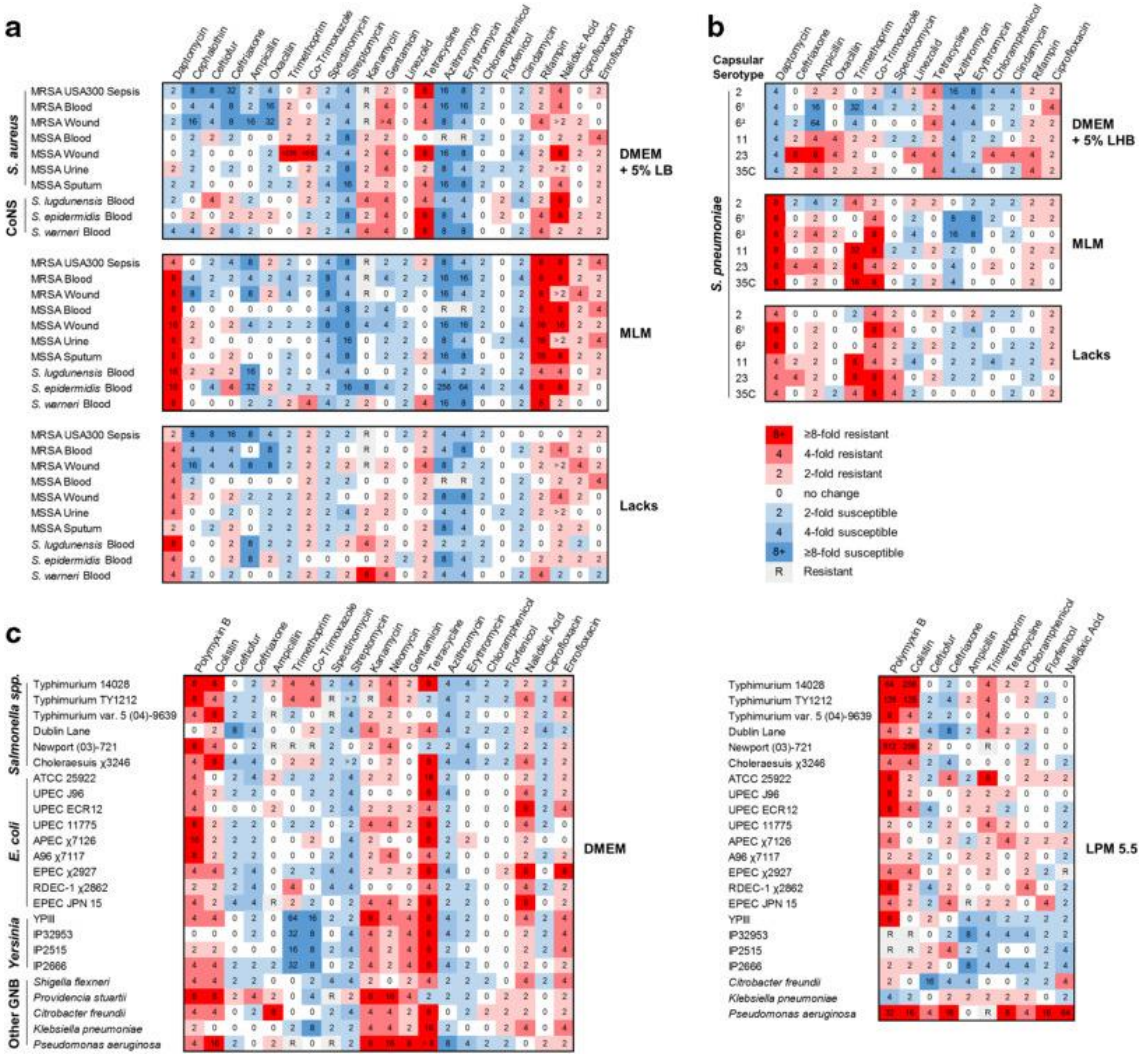

Figure 1. Comparison of pathogen-antibiotic combinations that exhibited altered MICs derived from host-mimicking media relative to standard MHB medium. A panel of antibiotics was screened for altered MICs against (a) *Staphylococcus* spp., (b) *S. pneumoniae*, and (c) Gram-negative bacteria when tested in Dulbecco's Modified Eagle Medium (DMEM), Lacks medium, modified Lacks medium (MLM), low-phosphate, low-magnesium medium (LPM pH 5.5) relative to standard MHB medium, according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2012a; Wiegand, Hilpert, & Hancock, 2008). Values depict the fold-change in MICs when derived in host-mimicking media relative to standard MHB medium (test/standard condition). Increased susceptibility depicted in blue; increased resistance depicted in red. MIC values were obtained from at least 6 independent determinations.

Table 1. AST in host-mimicking media identifies MICs that cross clinical breakpoint designations which advise on patient therapy.

Drug	Target	Pathogen	Host-mimicking media	Clinical breakpoint
Increased susceptibility				
Cephalothin	Cell wall	MRSA[1]	DMEM/MHB + $NaHCO_3$	R to S
Ceftriaxone	Cell wall	MRSA[1–3]	DMEM/MHB + $NaHCO_3$	R to S, I
Oxacillin	Cell wall	MRSA[3]	DMEM	R to S
Ampicillin	Cell wall	CoNS[1]; *SPN*[1,2]	MLM/DMEM	R, I to S
Trimethoprim	Folate	*SPN*[2]	DMEM	R to S
Azithromycin	Protein	CoNS[1,3]; *SPN*[1,2]	MLM/MHB + $NaHCO_3$	R to S, I
Erythromycin	Protein	MSSA[1,2]; CoNS[1]	DMEM/MLM/MHB + $NaHCO_3$	R, I to I, S
Streptomycin	Protein	MRSA[1]; MSSA[1–4]	DMEM/MLM	R, I to S
Decreased susceptibility				
Colistin	Membrane	*ST*; *PA*	DMEM	S to R
Daptomycin	Membrane	MRSA[2,3]; MSSA[1–4]; CoNS[1–3]	MLM	S to R
Ceftriaxone	Cell wall	*SPN*[3]	DMEM	S to R
Ampicillin	Cell wall	*SPN*[3]; CF	DMEM/MHB + $NaHCO_3$	S, I to R, I
Trimethoprim	Folate	MSSA[1]; *SPN*[4, 5]	DMEM/MLM	S to R
Co-Trimoxazole	Folate	MSSA[1]; *SPN*[4, 5]	DMEM/MLM	S to R, I
Gentamicin	Protein	*PA*	DMEM	S to R
Tetracycline	Protein	*KPN*; CF; *ST*; *SC*; *EC*[1–3]; *YP*[1–4]	DMEM/MHB + $NaHCO_3$	S to R, I
Enrofloxacin	DNA	*EC*[4]	DMEM	S to R

Depicted are pathogen-antibiotic combinations that exhibited altered MICs derived from host-mimicking media relative to standard MHB medium; and whose MICs crossed clinical breakpoint designations that are used to define isolates as susceptible (“S”), intermediate (“I”), or resistant (“R”), and advise on patient therapy. R to S refers to an “R” classification when tested for susceptibility in MHB medium but an “S” classification in host-mimicking media. MRSA[1–3] (USA 300; Blood; Wound); CoNS[1–3] (*S. epidermidis*; *S. lugdunensis*; *S. warneri*); *SPN*[1–5] (serotype 6; 6; 23; 11; 35C); MSSA[1–4] (Wound; Sputum; Urine; Blood); *ST* (*S.* Typhimurium); *PA* (*P. aeruginosa*); CF (*C. freundii*); *KPN* (*K. pneumoniae*); *SC* (*S.* Choleraesuis); *EC*[1–

[4] (*E. coli* ATCC 25922; UPEC J96; UPEC ATCC 11775; EPEC χ2927); *YP*[1–4] (YPIII; IP32953; IP2515; IP2666). Clinical breakpoint concentrations for listed drugs (Clinical and Laboratory Standards Institute, 2012b, 2013, 2014; European Committee on Antimicrobial Susceptibility Testing, 2016; Fuchs, Barry, & Brown, 1997; Landman, Georgescu, Martin, & Quale, 2008; Societe Francaise de Microbiologie, 2012).

Table 2a. Antimicrobial susceptibility testing in host-mimicking media (*Staphylococcus*)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

		Daptomycin MIC (µg/mL)				Cephalothin MIC (µg/mL)				Ceftiofur MIC (µg/mL)				Ceftriaxone MIC (µg/mL)			
Clinical Breakpoints[a]:		S ≤ 1; NS ≥ 2				S ≤ 8; I = 16; R ≥ 32[163]				S ≤ 2; I = 4; R ≥ 8[164]				S ≤ 8; I = 16-32; R ≥ 64[163]			
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	1	0.5	4	2	32	4	32	4	64	8	32	8	512	16	128	32
MT3302	MRSA Blood	0.5	0.5	4	2	8	2	2	2	16	4	8	4	128	16	64	32
MT3315	MRSA Wound	0.5	0.25	4	2	8	0.5	1	0.5	16	4	8	4	64	8	64	16
MT3305	MSSA Blood	0.5	0.5	4	2	0.5	0.25	0.5	0.25	1	2	1	1	4	2	4	4
MT3307	MSSA Wound	0.5	0.5	8	2	0.125	0.0625	0.25	0.25	1	1	1	1	2	2	4	4
MT3309	MSSA Urine	0.5	1	4	2	0.25	0.125	0.5	0.25	1	1	1	1	4	2	4	2
MT3314	MSSA Sputum	1	0.5	8	2	0.25	0.125	0.25	0.25	1	1	1	0.5	2	2	4	4
MT3317	CoN S. lugdunensis	0.25	0.125	4	2	0.5	0.5	1	0.5	0.5	2	1	0.5	2	4	4	4
MT3320	CoN S. epidermidis	0.5	0.5	8	2	0.125	0.25	0.125	0.125	0.5	0.5	0.125	0.5	1	2	4	2
MT3321	CoN S. warneri	0.5	0.125	4	2	0.125	0.03125	0.125	0.0625	0.25	0.5	0.25	0.25	2	0.5	2	1

		Ampicillin MIC (µg/mL)				Oxacillin MIC (µg/mL)				Trimethoprim MIC (µg/mL)				Co-Trimoxazole MIC (µg/mL)			
Clinical Breakpoints[a]:		S ≤ 0.25; R ≥ 0.5[163]				S ≤ 2; R ≥ 4				S ≤ 8; R ≥ 16				S ≤ 2/38; R ≥ 4/76			
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	512	256	64	64	64	16	128	16	2	2	1	1	0.0625/1.2	0.125/2.4	0.0625/1.2	0.125/2.4
MT3302	MRSA Blood	256	128	64	256	64	4	32	8	2	4	0.5	1	0.125/2.4	0.125/2.4	0.0625/1.2	0.25/4.8
MT3315	MRSA Wound	8	0.5	1	1	32	1	64	4	2	4	0.5	1	0.0625/1.2	0.125/2.4	0.0625/1.2	0.125/2.4
MT3305	MSSA Blood	2	2	2	2	0.25	0.25	0.25	0.25	1	2	1	1	0.0625/1.2	0.125/2.4	0.0625/1.2	0.125/2.4
MT3307	MSSA Wound	0.125	0.125	0.03125	0.0625	0.25	0.125	0.125	0.125	2	>512	1	1	0.125/2.4	>64/1216	0.0625/1.2	0.25/4.8
MT3309	MSSA Urine	0.125	0.125	0.125	0.125	0.25	0.125	0.25	0.125	1	1	1	0.5	0.0625/1.2	0.125/2.4	0.0625/1.2	0.125/2.4
MT3314	MSSA Sputum	0.125	0.125	0.125	0.125	0.25	0.125	0.25	0.125	2	1	1	1	0.0625/1.2	0.125/2.4	0.0625/1.2	0.125/2.4
MT3317	CoN S. lugdunensis	128	64	8	16	0.5	0.5	0.5	0.25	16	16	8	8	0.25/4.8	0.5/9.5	0.25/4.8	0.5/9.5
						Clinical Breakpoints: S ≤ 0.25; R ≥ 0.5											
MT3320	CoN S. epidermidis	8	16	0.25	1	0.125	0.25	0.25	0.25	1	1	0.5	0.5	0.25/4.8	0.125/2.4	0.125/2.4	0.25/4.8
MT3321	CoN S. warneri	0.03125	0.0156	0.0156	0.03125	0.125	0.125	0.0625	0.125	2	2	4	1	0.0625/1.2	0.125/2.4	0.25/4.8	0.125/2.4

		Spectinomycin MIC (µg/mL)				Streptomycin MIC (µg/mL)				Kanamycin MIC (µg/mL)				Gentamicin MIC (µg/mL)			
Clinical Breakpoints:						S ≤ 8; I = 16; R ≥ 32[165]				S ≤ 16; I = 32; R ≥ 64				S ≤ 4; I = 8; R ≥ 16			
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	128	64	32	64	16	4	2	8	> 512	> 512	> 512	> 512	1	2	0.5	1
MT3302	MRSA Blood	128	32	16	64	8	2	2	8	> 512	> 512	> 512	> 512	1	4	0.25	1
MT3315	MRSA Wound	128	64	16	64	4	1	1	8	> 512	> 512	512	> 512	128	>512	128	256
MT3305	MSSA Blood	128	64	32	64	16	2	2	8	8	16	4	8	1	2	0.25	1
MT3307	MSSA Wound	256	64	32	128	16	4	2	16	8	16	2	8	1	4	0.25	2
MT3309	MSSA Urine	128	64	32	64	32	4	2	8	4	8	4	8	0.5	2	0.25	1
MT3314	MSSA Sputum	256	64	64	128	32	2	4	16	4	8	4	8	1	2	0.5	1
MT3317	CoN S. lugdunensis	64	32	16	32	4	1	1	8	1	4	1	4	0.125	0.5	0.125	0.25
MT3320	CoN S. epidermidis	128	64	64	128	4	0.5	0.25	4	4	8	0.5	4	0.125	0.5	0.03125	0.25
MT3321	CoN S. warneri	64	32	16	32	4	1	2	8	1	4	2	8	0.125	0.5	0.125	0.5

		Linezolid MIC (µg/mL)				Tetracycline MIC (µg/mL)				Azithromycin MIC (µg/mL)				Erythromycin MIC (µg/mL)			
Clinical Breakpoints:		S ≤ 4; R ≥ 8				S ≤ 4; I = 8; R ≥ 16				S ≤ 2; I = 4; R ≥ 8				S ≤ 0.5; I = 1 - 4; R ≥ 8			
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	4	4	2	2	0.5	4	1	1	128	8	16	32	64	8	16	16
MT3302	MRSA Blood	2	2	1	2	0.5	2	0.25	1	128	8	8	32	128	8	8	16
MT3315	MRSA Wound	2	2	1	2	0.25	1	0.25	1	1	0.125	0.0625	0.125	0.25	0.0625	0.0625	0.125
MT3305	MSSA Blood	2	2	2	2	0.5	0.5	0.5	1	>512	>512	>512	>512	>512	>512	>512	>512
MT3307	MSSA Wound	2	2	1	2	0.5	4	0.5	1	2	0.125	0.125	0.25	1	0.125	0.0625	0.125
MT3309	MSSA Urine	2	2	1	2	4	8	4	4	1	0.125	0.125	0.25	0.5	0.125	0.125	0.125
MT3314	MSSA Sputum	2	2	1	2	0.5	2	1	1	2	0.125	0.125	0.25	1	0.125	0.125	0.25
MT3317	CoN S. lugdunensis	1	1	0.5	1	0.25	1	0.25	0.5	0.25	0.0625	0.0625	0.125	0.125	0.03125	0.0625	0.0625
MT3320	CoN S. epidermidis	2	2	1	1	0.5	4	0.5	1	256	32	1	32	128	32	2	32
MT3321	CoN S. warneri	2	2	1	2	0.5	4	2	1	256	32	16	64	256	32	32	64

		Chloramphenicol MIC (µg/mL)				Florfenicol MIC (µg/mL)				Clindamycin MIC (µg/mL)				Rifampin MIC (µg/mL)			
Clinical Breakpoints:		S ≤ 8; I = 16; R ≥ 32								S ≤ 0.5; I = 1 - 2; R ≥ 4				S ≤ 1; I = 2; R ≥ 4			
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	16	8	8	8	4	4	4	4	0.125	0.0625	0.0625	0.125	0.0078	0.0156	0.0625	0.0078
MT3302	MRSA Blood	8	4	4	8	4	4	4	4	0.25	0.125	0.0625	0.125	0.0078	0.0156	0.0625	0.0156
MT3315	MRSA Wound	8	8	4	4	4	4	4	4	0.125	0.125	0.0625	0.125	0.0078	0.03125	0.0625	0.0156
MT3305	MSSA Blood	16	8	8	8	4	4	4	4	0.25	0.125	0.125	0.125	0.0156	0.0156	0.125	0.0156
MT3307	MSSA Wound	16	16	8	8	4	4	4	4	0.25	0.0625	0.0625	0.125	0.0078	0.0156	0.125	0.0156
MT3309	MSSA Urine	8	4	8	8	8	4	4	4	0.25	0.125	0.0625	0.125	0.0078	0.0156	0.125	0.0156
MT3314	MSSA Sputum	8	4	8	8	4	4	4	4	0.25	0.125	0.125	0.125	0.0156	0.0156	0.25	0.0156
MT3317	CoN S. lugdunensis	8	8	4	4	2	4	2	2	0.125	0.03125	0.0625	0.0625	0.0078	0.0156	0.03125	0.0078
MT3320	CoN S. epidermidis	8	8	2	4	4	8	2	4	0.125	0.125	0.03125	0.125	0.0078	0.03125	0.0625	0.0156
MT3321	CoN S. warneri	8	8	8	8	4	4	4	4	0.125	0.0625	0.0625	0.0625	0.002	0.0078	0.0156	0.0078

		Nalidixic Acid MIC (µg/mL)				Ciprofloxacin MIC (µg/mL)				Enrofloxacin MIC (µg/mL)			
Clinical Breakpoints:						S ≤ 1; I = 2; R ≥ 4							
			DMEM +		Lacks		DMEM +		Lacks		DMEM +		Lacks
Strain #	Strain Name	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium	Ca-MHB	5% LB	MLM	Medium
MT3322	MRSA USA300	64	256	512	64	0.5	0.5	1	1	0.125	0.25	0.5	0.25
MT3302	MRSA Blood	64	256	512	256	0.25	0.25	0.5	0.5	0.25	0.25	0.5	0.25
MT3315	MRSA Wound	256	>512	>512	>512	8	16	32	32	4	4	8	8
MT3305	MSSA Blood	64	128	512	128	0.125	0.25	0.25	0.25	0.0625	0.25	0.25	0.25
MT3307	MSSA Wound	32	256	512	128	0.25	0.5	0.5	0.5	0.125	0.25	0.25	0.125
MT3309	MSSA Urine	256	>512	>512	>512	16	16	32	16	4	8	16	4
MT3314	MSSA Sputum	32	128	256	64	0.25	0.25	0.5	0.5	0.125	0.25	0.25	0.125
MT3317	CoN S. lugdunensis	64	512	256	128	0.25	0.25	0.25	0.125	0.125	0.25	0.25	0.125
MT3320	CoN S. epidermidis	32	256	256	64	0.125	0.25	0.25	0.25	0.125	0.25	0.125	0.25
MT3321	CoN S. warneri	256	512	512	128	0.25	0.5	0.25	0.25	0.25	0.5	0.25	0.125

MHB, DMEM, MLM and Lacks Medium MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[30] unless otherwise indicated

Table 2b. Antimicrobial susceptibility testing in host-mimicking media (*Streptococcus pneumoniae*)

		Daptomycin MIC (µg/mL)				Ceftriaxone MIC (µg/mL)				Ampicillin MIC (µg/mL)				Oxacillin MIC (µg/mL)			
Clinical Breakpoints[a]:		S ≤ 2; NS ≥4[166]				S ≤ 1; I = 2; R ≥ 4				S ≤ 0.5; I = 1-2; R ≥ 4[167]							
Strain	Capsular Serotype	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium
D39	2	0.25	0.0625	2	1	0.0156	0.0156	0.0078	0.0156	0.0156	0.03125	0.0039	0.0156	0.0625	0.125	0.03125	0.0625
Daw1	6	0.25	0.0625	2	2	0.5	0.5	0.5	0.5	1	0.0625	2	2	8	8	8	8
Daw19	6	0.25	0.0625	2	2	1	0.5	2	1	4	0.0625	16	8	8	8	16	8
Daw20	11	0.25	0.0625	2	1	0.0156	0.03125	0.0156	0.03125	0.0156	0.0625	0.03125	0.03125	0.0625	0.25	0.0625	0.0625
Daw2	23	0.25	0.0625	2	1	0.5	4	2	2	0.5	4	2	1	2	8	4	2
Daw25	35C	0.25	0.0625	2	1	0.0156	0.03125	0.0156	0.0156	0.0156	0.0625	0.03125	0.03125	0.0625	0.125	0.0625	0.03125

		Trimethoprim MIC (µg/mL)				Co-Trimoxazole MIC (µg/mL)				Spectinomycin MIC (µg/mL)				Linezolid MIC (µg/mL)			
Clinical Breakpoints:		S ≤ 2; R ≥ 4[168]				S ≤ 0.5/9.5; I = 1/19 - 2/38; R ≥ 4/76								S ≤ 2; NS ≥ 4			
Strain	Capsular Serotype	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium
D39	2	0.5	0.5	2	0.25	0.0625/1.2	0.125/2.4	0.125/2.4	0.25/4.8	32	8	32	64	0.5	1	1	0.5
Daw1	6	64	2	64	64	4/76	1/19	16/304	32/608	16	8	16	64	1	0.5	0.5	1
Daw19	6	64	16	64	64	4/76	4/76	32/604	16/304	16	16	16	32	2	2	0.5	1
Daw20	11	1	2	32	8	0.25/4.8	0.5/9.5	2/38	1/19	32	16	16	64	2	1	1	0.5
Daw2	23	4	8	32	32	2/38	2/38	8/152	16/304	16	16	32	64	0.5	2	0.5	0.5
Daw25	35C	1	2	16	4	0.125/2.4	0.25/4.8	1/19	1/19	16	16	16	64	1	1	0.5	0.5

		Tetracycline MIC (µg/mL)				Azithromycin MIC (µg/mL)				Erythromycin MIC (µg/mL)				Chloramphenicol MIC (µg/mL)			
Clinical Breakpoints:		S ≤ 1; I = 2; R ≥ 4				S ≤ 0.5; I = 1; R ≥ 2				S ≤ 0.25; I = 0.5; R ≥ 1				S ≤ 4; R ≥ 8			
Strain	Capsular Serotype	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium
D39	2	0.125	0.5	0.25	0.25	0.0625	0.0039	0.0625	0.0625	0.03125	0.0039	0.0156	0.0625	4	1	2	2
Daw1	6	0.5	1	0.5	0.5	8	2	1	4	8	2	1	2	4	2	2	4
Daw19	6	0.25	1	0.25	0.5	8	2	0.5	4	8	2	1	4	2	2	2	2
Daw20	11	0.25	0.5	0.5	0.25	0.0625	0.0156	0.03125	0.03125	0.03125	0.0156	0.03125	0.0156	4	2	4	1
Daw2	23	0.125	0.5	0.125	0.25	0.0625	0.0156	0.0156	0.03125	0.03125	0.0156	0.03125	0.0156	1	4	2	1
Daw25	35C	0.125	0.5	0.25	0.125	0.0625	0.0156	0.0156	0.0625	0.03125	0.0078	0.03125	0.03125	2	2	2	2

		Clindamycin MIC (µg/mL)				Rifampin MIC (µg/mL)				Ciprofloxacin MIC (µg/mL)			
Clinical Breakpoints:		S ≤ 0.25; I = 0.5; R ≥ 1				S ≤ 1; I = 2; R ≥ 4				S ≤ 0.125; I = 0.25-2; R ≥ 4[167]			
Strain	Capsular Serotype	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium	Ca-MHB + 5% LHB	DMEM + 5% LHB	MLM	Lacks Medium
D39	2	0.03125	0.0078	0.0156	0.0156	0.0156	0.03125	0.03125	0.0156	0.5	1	1	1
Daw1	6	0.0625	0.03125	0.0625	0.0625	0.0156	0.0156	0.03125	0.0156	0.5	2	1	1
Daw19	6	0.0625	0.03125	0.03125	0.03125	0.0156	0.03125	0.0156	0.0078	0.5	1	0.5	1
Daw20	11	0.0625	0.0625	0.0625	0.03125	0.03125	0.0625	0.0625	0.0156	1	2	2	2
Daw2	23	0.0156	0.0625	0.0156	0.0156	0.0156	0.0625	0.03125	0.0078	1	2	1	1
Daw25	35C	0.0625	0.03125	0.0625	0.03125	0.0156	0.0625	0.03125	0.0156	0.5	1	0.5	1

MHB, DMEM, MLM and Lacks Medium MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[30] unless otherwise indicated

Table 2c. Antimicrobial susceptibility testing in host-mimicking media (*Salmonella*)

Clinical Breakpoints[a]:	Polymyxin B (µg/mL) S ≤ 2, R ≥ 4[ISO]						Colistin Sulfate (µg/mL) S ≤ 2, R ≥ 4[CLSI]						Ceftiofur (µg/mL) S ≤ 2, I = 4, R ≥ 8[CLSI]						Ceftriaxone (µg/mL) S ≤ 1, I = 2, R ≥ 4						Ampicillin (µg/mL) S ≤ 8, I = 16, R ≥ 32					
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
S. Typhimurium 14028	0.5	0.25	2	1	1	128	0.5	0.5	4	1	0.5	256	1	0.5	0.5	1	1	1	0.0625	0.125	0.0625	0.125	0.0625	0.0625	2	2	4	1	2	1
S. Typhimurium TY1212	0.5	0.25	2	1	0.5	128	0.5	0.5	2	1	0.5	128	1	1	0.5	2	1	1	0.125	0.25	0.125	0.5	0.125	0.125	4	4	4	1	2	1
S. Typhimurium str. 5 (DI)-9639	0.5	0.25	1	0.5	0.5	4	0.5	0.25	2	1	0.5	4	1	1	0.5	2	1	1	0.125	0.125	0.0625	0.5	0.125	0.25	2048	>2048	>2048	512	1024	256
S. Dublin Lane	2	2	2	4	16	128	2	4	8	16	16	256	0.5	1	0.125	2	1	1	0.0625	0.0625	0.0156	0.5	0.0625	0.0625	2	1	1	2	4	2
S. Newport (D)-721	0.5	0.25	2	0.5	0.5	256	0.5	0.5	2	1	0.5	256	64	128	128	8	16	4	64	128	64	32	32	16	1024	2048	>2048	512	512	256
S. Choleraesuis χ3246	0.5	0.25	1	0.5	0.5	2	0.5	0.25	2	0.5	0.5	2	1	2	0.5	1	1	0.5	0.0625	0.125	0.03125	0.25	0.0625	0.0625	2	2	2	1	2	1

Clinical Breakpoints:	Trimethoprim (µg/mL) S ≤ 8, R ≥ 16						Co-trimoxazole (µg/mL) S ≤ 2/38, R ≥ 4/76					Spectinomycin (µg/mL) S ≤ 32; I = 64-128; R ≥ 256[ISO]					Streptomycin (µg/mL) S ≤ 8; I = 16, R ≥ 32[ISO]					Kanamycin (µg/mL) S ≤ 16, I = 32, R ≥ 64					
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
S. Typhimurium 14028	0.25	0.25	1	1	1	16	0.0625/1.2	0.25/4.8	NA	NA	NA	64	32	NA	NA	NA	32	8	NA	NA	NA	4	8	NA	NA	NA
S. Typhimurium TY1212	0.25	0.5	2	1	1	16	0.25/4.8	1/19	NA	NA	NA	>1024	>1024	NA	NA	NA	>1024	512	NA	NA	NA	2048	>2048	NA	NA	NA
S. Typhimurium str. 5 (DI)-9639	0.25	1	0.5	1	1	16	0.25/4.8	0.25/4.8	NA	NA	NA	>1024	>1024	NA	NA	NA	256	64	NA	NA	NA	4	8	NA	NA	NA
S. Dublin Lane	0.25	0.5	0.5	1	1	16	0.125/2.4	0.25/4.8	NA	NA	NA	64	32	NA	NA	NA	32	8	NA	NA	NA	2	8	NA	NA	NA
S. Newport (D)-721	>128	>256	>256	>128	>128	>128	>64/1216	>64/1216	NA	NA	NA	128	64	NA	NA	NA	1024	1024	NA	NA	NA	4	8	NA	NA	NA
S. Choleraesuis χ3246	0.125	0.25	0.5	0.5	0.25	1	0.25/4.8	0.5/9.5	NA	NA	NA	64	32	NA	NA	NA	>1024	512	NA	NA	NA	4	4	NA	NA	NA

Clinical Breakpoints:	Neomycin (µg/mL) S ≤ 8, I = 16, R ≥ 32[CLSI]					Gentamicin (µg/mL) S ≤ 4, I = 8, R ≥ 16					Tetracycline (µg/mL) S ≤ 4, I = 8, R ≥ 16						Azithromycin (µg/mL) S ≤ 16, R ≥ 32[EC]					Erythromycin (µg/mL)				
Strain Name	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
S. Typhimurium 14028	2	8	NA	NA	NA	1	2	NA	NA	NA	2	1	8	1	1	1	8	2	NA	NA	NA	128	32	NA	NA	NA
S. Typhimurium TY1212	256	1024	NA	NA	NA	1	2	NA	NA	NA	256	256	128	512	128	256	8	2	NA	NA	NA	128	64	NA	NA	NA
S. Typhimurium str. 5 (DI)-9639	2	4	NA	NA	NA	1	1	NA	NA	NA	64	64	64	64	32	32	8	2	NA	NA	NA	128	64	NA	NA	NA
S. Dublin Lane	1	2	NA	NA	NA	0.5	1	NA	NA	NA	2	1	4	1	1	1	4	1	NA	NA	NA	64	32	NA	NA	NA
S. Newport (D)-721	2	8	NA	NA	NA	1	1	NA	NA	NA	128	256	128	256	64	128	4	2	NA	NA	NA	128	32	NA	NA	NA
S. Choleraesuis χ3246	1	2	NA	NA	NA	1	1	NA	NA	NA	2	1	8	1	1	0.5	8	2	NA	NA	NA	128	32	NA	NA	NA

Clinical Breakpoints:	Chloramphenicol (µg/mL) S ≤ 8, I = 16, R ≥ 32						Florfenicol (µg/mL) S ≤ 4, I = 8, R ≥ 16[EM]						Nalidixic Acid (µg/mL) S ≤ 16, R ≥ 32						Ciprofloxacin (µg/mL) S ≤ 0.0625, I = 0.125-0.5, R ≥ 1					Enrofloxacin (µg/mL) S ≤ 0.5; I = 1; R ≥ 2[EC]				
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
S. Typhimurium 14028	4	8	4	2	2	2	4	8	4	4	4	4	4	4	8	2	2	1	0.0156	0.0078	NA	NA	NA	0.03125	0.0625	NA	NA	NA
S. Typhimurium TY1212	512	512	256	256	64	64	256	512	256	128	128	32	4	4	16	2	2	1	0.0156	0.0078	NA	NA	NA	0.03125	0.125	NA	NA	NA
S. Typhimurium str. 5 (DI)-9639	256	256	256	256	64	64	32	64	64	16	32	16	4	4	8	2	2	1	0.0156	0.0078	NA	NA	NA	0.03125	0.0625	NA	NA	NA
S. Dublin Lane	4	4	2	2	2	2	4	4	2	4	4	4	4	4	8	1	4	1	0.0156	0.0039	NA	NA	NA	0.0625	0.03125	NA	NA	NA
S. Newport (D)-721	256	256	256	256	32	16	256	256	128	128	32	16	4	4	8	2	2	1	0.0156	0.0078	NA	NA	NA	0.03125	0.0625	NA	NA	NA
S. Choleraesuis χ3246	4	4	2	2	2	2	4	4	2	4	4	4	4	4	16	2	4	1	0.0156	0.0078	NA	NA	NA	0.0625	0.125	NA	NA	NA

MHB and DMEM MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[M] unless otherwise indicated

Table 2d. Antimicrobial susceptibility testing in host-mimicking media (*Escherichia coli*)

Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH5.5	LPM pH7	LPM pH5.5
Polymyxin B (µg/mL)						
Clinical breakpoints[a]:		S ≤ 2, R ≥ 4[144]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	0.25	1	0.5	0.5	4
UPEC J96	0.5	0.25	1	0.25	0.5	2
UPEC EC R12	1	0.25	1	0.25	0.5	1
UPEC ATCC 11775	0.5	0.125	1	0.5	1	2
APEC χ7126	0.5	0.125	2	0.5	0.5	2
A95 χ7117	0.5	0.125	1	0.5	0.5	1
EPEC χ2927	0.5	0.25	1	0.25	0.5	1
RDEC-1 χ2862	0.5	0.25	0.5	0.125	0.5	1
EPEC JPN 15	0.5	0.25	1	0.25	0.5	1
Colistin Sulfate (µg/mL)						
Clinical breakpoints[a]:		S ≤ 2, R ≥ 4[145]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	1	1	4	4	32
UPEC J96	0.5	1	1	2	4	32
UPEC EC R12	0.5	1	1	2	4	32
UPEC ATCC 11775	0.5	1	1	2	4	16
APEC χ7126	0.5	1	2	2	4	16
A95 χ7117	0.5	1	1	1	4	16
EPEC χ2927	0.25	1	0.25	1	4	4
RDEC-1 χ2862	0.5	1	1	1	4	16
EPEC JPN 15	0.5	1	1	1	1	4
Ceftriaxone (µg/mL)						
Clinical breakpoints[a]:		S ≤ 2, I = 4, R ≥ 8[141]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	0.5	0.25	2	0.25	0.5
UPEC J96	0.5	0.5	0.25	1	0.25	0.5
UPEC EC R12	0.5	0.5	0.5	1	0.5	0.5
UPEC ATCC 11775	0.25	0.5	0.25	1	0.25	0.5
APEC χ7126	0.25	0.25	0.125	0.5	0.125	0.25
A95 χ7117	0.25	0.25	0.125	1	0.25	0.5
EPEC χ2927	0.5	0.5	0.25	1	0.25	0.5
RDEC-1 χ2862	0.5	0.5	0.25	1	0.25	0.25
EPEC JPN 15	0.5	2	0.5	1	0.5	0.5
Cefotaxime (µg/mL)						
Clinical breakpoints[a]:		S ≤ 1, I = 2, R ≥ 4				
Seattle 1946; O6:K5:H1 ATCC 25922	0.125	0.03125	0.125	0.015625	0.0625	
UPEC J96	0.0625	0.0625	0.125	0.015625	0.03125	
UPEC EC R12	0.0625	0.0625	0.125	0.015625	0.03125	
UPEC ATCC 11775	0.0625	0.03125	0.125	0.0078125	0.03125	
APEC χ7126	0.03125	0.0156	0.0625	0.0078125	0.03125	
A95 χ7117	0.0625	0.03125	0.0625	0.015625	0.015625	
EPEC χ2927	0.0625	0.0156	0.0625	0.0078125	0.015625	
RDEC-1 χ2862	0.125	0.03125	0.125	0.0078125	0.015625	
EPEC JPN 15	0.125	0.03125	0.125	0.015625	0.0625	
Ampicillin (µg/mL)						
Clinical breakpoints[a]:		S ≤ 8, I = 16, R ≥ 32				
Seattle 1946; O6:K5:H1 ATCC 25922	4	8	4	2	1	
UPEC J96	4	4	2	1	1	
UPEC EC R12	4	8	2	1	1	
UPEC ATCC 11775	4	4	2	1	0.5	
APEC χ7126	2	2	2	1	0.5	
A95 χ7117	4	4	2	1	1	
EPEC χ2927	4	4	2	1	0.5	
RDEC-1 χ2862	8	8	4	1	0.5	
EPEC JPN 15	>2048	>2048	>2048	>2048	>2048	
Trimethoprim (µg/mL)						
Clinical breakpoints:		S ≤ 8, R ≥ 16				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	1	0.5	4	0.5	16
UPEC J96	0.5	0.5	0.5	4	1	16
UPEC EC R12	0.25	0.5	0.5	4	1	16
UPEC ATCC 11775	0.5	1	0.5	4	1	16
APEC χ7126	0.125	0.25	0.25	2	1	16
A95 χ7117	0.125	0.25	0.25	4	0.5	16
EPEC χ2927	0.125	0.25	0.125	4	0.5	16
RDEC-1 χ2862	0.125	0.25	1	4	1	16
EPEC JPN 15	0.25	0.25	0.5	4	0.5	16
Co-trimoxazole (µg/mL)						
Clinical breakpoints:		S ≤ 2/38, R ≥ 4/76				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	0.06/1.2	0.03/1.2/0.6	NA	NA	NA
UPEC J96	0.5	0.06/1.2	0.03/1.2/0.6	NA	NA	NA
UPEC EC R12	0.5	0.06/1.2	0.03/1.2/0.6	NA	NA	NA
UPEC ATCC 11775	0.5	0.125/2.4	0.06/2.5/1.2	NA	NA	NA
APEC χ7126	0.5	0.03/1.2/0.6	0.06/2.5/1.2	NA	NA	NA
A95 χ7117	0.5	0.03/1.2/0.6	0.03/1.2/0.6	NA	NA	NA
EPEC χ2927	0.5	0.06/2.5/1.2	0.03/1.2/0.6	NA	NA	NA
RDEC-1 χ2862	0.5	0.03/1.2/0.6	0.03/1.2/0.6	NA	NA	NA
EPEC JPN 15	0.5	0.06/2.5/1.2	0.03/1.2/0.6	NA	NA	NA
Spectinomycin (µg/mL)						
Clinical breakpoints:		S ≤ 32; I = 64-128; R ≥ 256[174]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	32	16	NA	NA	NA
UPEC J96	0.5	32	16	NA	NA	NA
UPEC EC R12	0.25	16	8	NA	NA	NA
UPEC ATCC 11775	0.5	16	16	NA	NA	NA
APEC χ7126	0.125	16	16	NA	NA	NA
A95 χ7117	0.125	32	16	NA	NA	NA
EPEC χ2927	0.125	32	8	NA	NA	NA
RDEC-1 χ2862	0.125	16	4	NA	NA	NA
EPEC JPN 15	0.25	16	16	NA	NA	NA
Streptomycin (µg/mL)						
Clinical breakpoints:		S ≤ 8; I = 16, R ≥ 32[146]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	8	2	NA	NA	NA
UPEC J96	0.5	4	1	NA	NA	NA
UPEC EC R12	0.25	8	2	NA	NA	NA
UPEC ATCC 11775	0.5	4	2	NA	NA	NA
APEC χ7126	0.125	8	2	NA	NA	NA
A95 χ7117	0.125	8	2	NA	NA	NA
EPEC χ2927	0.125	4	1	NA	NA	NA
RDEC-1 χ2862	0.125	4	1	NA	NA	NA
EPEC JPN 15	0.25	8	4	NA	NA	NA
Kanamycin (µg/mL)						
Clinical breakpoints:		S ≤ 16, I = 32, R ≥ 64				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	4	8	NA	NA	NA
UPEC J96	0.5	4	4	NA	NA	NA
UPEC EC R12	0.25	4	8	NA	NA	NA
UPEC ATCC 11775	0.5	2	8	NA	NA	NA
APEC χ7126	0.5	4	8	NA	NA	NA
A95 χ7117	0.5	4	8	NA	NA	NA
EPEC χ2927	0.5	1	2	NA	NA	NA
RDEC-1 χ2862	0.5	2	2	NA	NA	NA
EPEC JPN 15	0.5	2	8	NA	NA	NA
Neomycin (µg/mL)						
Clinical breakpoints:		S ≤ 8, I = 16, R ≥ 32[171]				
Seattle 1946; O6:K5:H1 ATCC 25922	2	4	NA	NA	NA	
UPEC J96	2	2	NA	NA	NA	
UPEC EC R12	2	4	NA	NA	NA	
UPEC ATCC 11775	1	4	NA	NA	NA	
APEC χ7126	2	2	NA	NA	NA	
A95 χ7117	1	4	NA	NA	NA	
EPEC χ2927	0.5	1	NA	NA	NA	
RDEC-1 χ2862	1	1	NA	NA	NA	
EPEC JPN 15	1	4	NA	NA	NA	
Gentamicin (µg/mL)						
Clinical breakpoints:		S ≤ 4, I = 8, R ≥ 16				
Seattle 1946; O6:K5:H1 ATCC 25922	1	1	NA	NA	NA	
UPEC J96	1	1	NA	NA	NA	
UPEC EC R12	0.5	1	NA	NA	NA	
UPEC ATCC 11775	0.5	1	NA	NA	NA	
APEC χ7126	1	1	NA	NA	NA	
A95 χ7117	1	1	NA	NA	NA	
EPEC χ2927	0.25	0.5	NA	NA	NA	
RDEC-1 χ2862	0.5	0.5	NA	NA	NA	
EPEC JPN 15	0.5	1	NA	NA	NA	
Tetracycline (µg/mL)						
Clinical breakpoints:		S ≤ 4, I = 8, R ≥ 16				
Seattle 1946; O6:K5:H1 ATCC 25922	0.5	0.5	8	0.5	0.5	0.5
UPEC J96	1	1	8	0.5	0.5	0.5
UPEC EC R12	1	2	8	1	1	0.5
UPEC ATCC 11775	1	1	8	0.5	0.5	0.5
APEC χ7126	1	0.5	4	0.5	0.25	0.5
A95 χ7117	1	1	4	1	0.5	0.5
EPEC χ2927	1	0.5	4	1	0.5	0.5
RDEC-1 χ2862	1	2	8	1	0.5	0.5
EPEC JPN 15	1	0.5	4	1	0.25	0.5
Azithromycin (µg/mL)						
Clinical breakpoints:		S ≤ 16, R ≥ 32[147]				
Seattle 1946; O6:K5:H1 ATCC 25922	4	2	NA	NA	NA	
UPEC J96	4	1	NA	NA	NA	
UPEC EC R12	4	2	NA	NA	NA	
UPEC ATCC 11775	4	2	NA	NA	NA	
APEC χ7126	4	2	NA	NA	NA	
A95 χ7117	4	2	NA	NA	NA	
EPEC χ2927	4	1	NA	NA	NA	
RDEC-1 χ2862	8	4	NA	NA	NA	
EPEC JPN 15	4	2	NA	NA	NA	
Erythromycin (µg/mL)						
Clinical breakpoints:						
Seattle 1946; O6:K5:H1 ATCC 25922	64	64	NA	NA	NA	
UPEC J96	64	64	NA	NA	NA	
UPEC EC R12	64	64	NA	NA	NA	
UPEC ATCC 11775	64	64	NA	NA	NA	
APEC χ7126	32	64	NA	NA	NA	
A95 χ7117	64	64	NA	NA	NA	
EPEC χ2927	32	32	NA	NA	NA	
RDEC-1 χ2862	256	128	NA	NA	NA	
EPEC JPN 15	64	32	NA	NA	NA	
Chloramphenicol (µg/mL)						
Clinical breakpoints:		S ≤ 8, I = 16, R ≥ 32				
Seattle 1946; O6:K5:H1 ATCC 25922	4	4	4	2	2	2
UPEC J96	4	8	8	4	2	2
UPEC EC R12	4	8	8	4	2	2
UPEC ATCC 11775	4	4	4	4	2	2
APEC χ7126	4	4	4	2	1	1
A95 χ7117	4	4	4	2	2	2
EPEC χ2927	4	4	4	2	2	2
RDEC-1 χ2862	8	8	8	2	1	1
EPEC JPN 15	4	8	8	4	2	2
Florfenicol (µg/mL)						
Clinical breakpoints:		S ≤ 16, R ≥ 32[197]				
Seattle 1946; O6:K5:H1 ATCC 25922	8	8	8	8	2	4
UPEC J96	8	8	8	8	4	4
UPEC EC R12	8	8	8	8	4	4
UPEC ATCC 11775	8	8	8	8	4	4
APEC χ7126	4	4	4	8	2	4
A95 χ7117	4	4	4	8	2	4
EPEC χ2927	4	4	4	8	4	4
RDEC-1 χ2862	8	8	8	8	2	2
EPEC JPN 15	8	8	8	8	2	8
Nalidixic Acid (µg/mL)						
Clinical breakpoints:		S ≤ 16, R ≥ 32				
Seattle 1946; O6:K5:H1 ATCC 25922	2	4	2	0.5	1	
UPEC J96	128	512	128	32	32	
UPEC EC R12	64	512	64	64	32	
UPEC ATCC 11775	2	8	2	1	0.5	
APEC χ7126	2	4	1	0.5	0.5	
A95 χ7117	2	4	2	1	0.5	
EPEC χ2927	256	2048	>512	256	>512	
RDEC-1 χ2862	4	16	4	2	1	
EPEC JPN 15	256	2048	>512	>512	>512	
Ciprofloxacin (µg/mL)						
Clinical breakpoints:		S ≤ 1, I = 2, R ≥ 4				
Seattle 1946; O6:K5:H1 ATCC 25922	0.0078	0.0039	NA	NA	NA	
UPEC J96	0.125	0.0625	NA	NA	NA	
UPEC EC R12	0.125	0.0625	NA	NA	NA	
UPEC ATCC 11775	0.0156	0.0078	NA	NA	NA	
APEC χ7126	0.0078	0.0078	NA	NA	NA	
A95 χ7117	0.0078	0.0039	NA	NA	NA	
EPEC χ2927	0.25	0.25	NA	NA	NA	
RDEC-1 χ2862	0.0156	0.0078	NA	NA	NA	
EPEC JPN 15	0.125	0.125	NA	NA	NA	
Enrofloxacin (µg/mL)						
Clinical breakpoints:		S ≤ 0.5; I = 1; R ≥ 2[147]				
Seattle 1946; O6:K5:H1 ATCC 25922	0.0078	0.0156	NA	NA	NA	
UPEC J96	0.25	0.5	NA	NA	NA	
UPEC EC R12	0.125	0.5	NA	NA	NA	
UPEC ATCC 11775	0.0156	0.0156	NA	NA	NA	
APEC χ7126	0.0078	0.0078	NA	NA	NA	
A95 χ7117	0.0078	0.0156	NA	NA	NA	
EPEC χ2927	0.25	2	NA	NA	NA	
RDEC-1 χ2862	0.0156	0.03125	NA	NA	NA	
EPEC JPN 15	0.25	0.5	NA	NA	NA	

MHB and DMEM MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are reference from the CLSI 2014 Twenty-fourth Informational Supplement[148] unless otherwise indicated

Table 2e. Antimicrobial susceptibility testing in host-mimicking media (*Yersinia pseudotuberculosis*)

	Polymyxin B (µg/mL)						Colistin Sulfate (µg/mL)						Cefiofur (µg/mL)						Ceftiaxone (µg/mL)						Ampicillin (µg/mL)					
Clinical Breakpoints*:	S ≤ 2, R ≥ 4[189]						S ≤ 2, R ≥ 4[187]						S ≤ 2, I = 4, R ≥ 8[194]						S ≤ 1, I = 2, R ≥ 4						S ≤ 8, I = 16, R ≥ 32					
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Y. pseudotuberculosis YPIIIpIB1	0.25	0.25	1	0.25	1	8	0.5	0.25	1	1	2	4	0.5	0.125	0.125	1	0.125	0.5	0.03125	0.03125	0.0156	0.0625	0.0156	0.03125	1	0.25	0.25	2	2	1
Y. pseudotuberculosis IP32953	256	128	128	>512	512	256	256	256	256	>512	512	512	0.25	0.125	0.125	1	0.25	1	0.015625	0.03125	0.0156	0.0625	0.03125	0.0625	1	0.25	0.25	4	4	2
Y. pseudotuberculosis IP2515	128	64	128	>512	>512	512	256	128	256	>512	1024	1024	0.25	0.125	0.125	1	0.25	2	0.03125	0.0156	0.0156	0.0625	0.0156	0.125	1	0.25	0.25	4	2	4
Y. pseudotuberculosis IP2666	0.25	0.25	1	0.25	0.5	1	0.5	0.25	1	1	0.5	2	0.25	0.125	0.0625	1	0.125	1	0.015625	0.0156	0.0078	0.0625	0.0156	0.0625	1	0.25	0.125	4	4	2

	Trimethoprim (µg/mL)						Co-trimoxazole (µg/mL)					Spectinomycin (µg/mL)						Streptomycin (µg/mL)						Kanamycin (µg/mL)					
Clinical Breakpoints:	S ≤ 8, R ≥ 16						S ≤ 2/38, R ≥ 4/76					S ≤ 32; I = 64-128; R ≥ 256[110]						S ≤ 8; I = 16, R ≥ 32[185]						S ≤ 16, I = 32, R ≥ 64					
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Y. pseudotuberculosis YPIIIpIB1	1	4	0.0625	64	0.5	8	0.25/4.8	0.0156/0.3	NA	NA	NA	16	8	NA	NA	NA	0.5	1	0.5	NA	NA	NA	0.25	0.25	2	NA	NA	NA
Y. pseudotuberculosis IP32953	1	2	0.0625	64	1	16	0.125/2.4	0.0156/0.3	NA	NA	NA	16	16	NA	NA	NA	4	4	1	NA	NA	NA	1	1	4	NA	NA	NA
Y. pseudotuberculosis IP2515	1	1	0.0625	64	0.5	8	0.125/2.4	0.0156/0.3	NA	NA	NA	16	8	NA	NA	NA	2	2	0.5	NA	NA	NA	0.5	0.5	2	NA	NA	NA
Y. pseudotuberculosis IP2666	1	2	0.0625	64	2	32	0.125/2.4	0.0156/0.3	NA	NA	NA	16	16	NA	NA	NA	1	2	1	NA	NA	NA	0.5	1	4	NA	NA	NA

	Neomycin (µg/mL)						Gentamicin (µg/mL)					Tetracycline (µg/mL)						Azithromycin (µg/mL)					Erythromycin (µg/mL)					
Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32[171]						S ≤ 4, I = 8, R ≥ 16					S ≤ 4, I = 8, R ≥ 16						S ≤ 16, R ≥ 32[187]											
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Y. pseudotuberculosis YPIIIpIB1	1	0.25	1	NA	NA	NA	0.125	0.5	NA	NA	NA	2	1	8	2	1	0.5	8	2	NA	NA	NA	64	32	NA	NA	NA
Y. pseudotuberculosis IP32953	2	1	2	NA	NA	NA	0.5	2	NA	NA	NA	2	1	8	2	2	0.5	8	2	NA	NA	NA	64	32	NA	NA	NA
Y. pseudotuberculosis IP2515	1	0.5	1	NA	NA	NA	0.25	1	NA	NA	NA	2	1	8	1	2	1	4	2	NA	NA	NA	64	64	NA	NA	NA
Y. pseudotuberculosis IP2666	1	1	2	NA	NA	NA	0.25	1	NA	NA	NA	2	1	8	2	2	0.5	8	2	NA	NA	NA	64	32	NA	NA	NA

	Chloramphenicol (µg/mL)						Florfenicol (µg/mL)						Nalidixic Acid (µg/mL)						Ciprofloxacin (µg/mL)						Enrofloxacin (µg/mL)					
Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32												S ≤ 16, R ≥ 32						S ≤ 1, I = 2, R ≥ 4						S ≤ 0.5; I = 1; R ≥ 2[189]					
Strain Name	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	MHB Agar	Ca-MHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Y. pseudotuberculosis YPIIIpIB1	8	8	8	8	4	2	4	4	4	8	4	4	0.5	0.5	2	0.5	0.5	0.25	0.0078	0.0078	0.0039	NA	NA	NA	0.0039	0.0039	0.0156	NA	NA	NA
Y. pseudotuberculosis IP32953	4	8	8	8	4	2	4	4	4	8	4	4	0.5	1	2	0.5	0.5	0.25	0.0156	0.0156	0.0078	NA	NA	NA	0.03125	0.0078	0.03125	NA	NA	NA
Y. pseudotuberculosis IP2515	8	4	4	8	8	8	4	4	4	8	8	8	0.5	1	2	1	1	0.5	0.0156	0.0156	0.0078	NA	NA	NA	0.0078	0.0039	0.0156	NA	NA	NA
Y. pseudotuberculosis IP2666	8	8	8	8	8	2	4	4	4	8	4	4	0.5	1	2	0.5	1	0.25	0.0078	0.0078	0.0078	NA	NA	NA	0.0078	0.0078	0.0156	NA	NA	NA

MHB and DMEM MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

*All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[25] unless otherwise indicated

Table 2f. Antimicrobial susceptibility testing in host-mimicking media (Misc Gram-negative)

			Polymyxin B (µg/mL)					Colistin Sulfate (µg/mL)					Cerilotur (µg/mL)					Ceftriaxone (µg/mL)					Ampicillin (µg/mL)				
	Clinical Breakpoints[a]:		S ≤ 2, R ≥ 4[66]					S ≤ 2, R ≥ 4[67]					S ≤ 2, I = 4, R ≥ 8[104]					S ≤ 1, I = 2, R ≥ 4					S ≤ 8, I = 16, R ≥ 32				
	Strain	Strain Name	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Enterobacteriaceae	MTI942	*Shigella flexneri* ATCC 29903	0.25	1	RA	RA	RA	0.25	1	RA	RA	RA	0.25	0.125	RA	RA	RA	0.00125	0.0195	RA	RA	RA	2	2	RA	RA	RA
	MTI944	*Providencia stuartii* ATCC 29914	32	256	RA	RA	RA	64	512	RA	RA	RA	0.25	0.5	RA	RA	RA	0.0195	0.0625	RA	RA	RA	32	64	RA	RA	RA
	MTI946	*Klebsiella pneumoniae* ATCC 13883	0.5	2	1	4	16	0.5	2	2	4	16	0.5	0.5	256	0.5	16	0.125	0.0625	8	0.0625	1	16	128	16	32	8
	MTI947	*Klebsiella pneumoniae* ATCC 13883	8	16	128	128	512	16	16	128	128	512	1	1	2	0.5	1	0.125	0.125	0.125	0.00125	0.0625	256	256	256	128	256
	Clinical Breakpoints:		S ≤ 2, I = 4, R ≥ 8					S ≤ 2, I = 4, R ≥ 8					S ≤ 2, I = 4, R ≥ 8[68]														
	MTI945	*Pseudomonas aeruginosa* ATCC 10145	2	8	1	2	32	1	16	1	2	32	64	32	64	4	16	8	8	16	0.5	16	128	256	512	16	64

			Trimethoprim (µg/mL)					Co-trimoxazole (µg/mL)					Spectinomycin (µg/mL)					Streptomycin (µg/mL)					Kanamycin (µg/mL)				
	Clinical Breakpoints:		S ≤ 8, R ≥ 16					S ≤ 2/38, R ≥ 4/76					S ≤ 32; I = 64–128; R ≥ 256[170]					S ≤ 8, I = 16, R ≥ 32[66]					S ≤ 16, I = 32, R ≥ 64				
	Strain	Strain Name	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Enterobacteriaceae	MTI942	*Shigella flexneri* ATCC 29903	0.25	0.25	RA	RA	RA	0.25/4.8	0.125/2.4	RA	RA	RA	64	16	RA	RA	RA	8	2	RA	RA	RA	4	8	RA	RA	RA
	MTI944	*Providencia stuartii* ATCC 29914	2	2	RA	RA	RA	0.25/4.8	0.0625/1.2	RA	RA	RA	>512	>512	RA	RA	RA	64	128	RA	RA	RA	0.5	4	RA	RA	RA
	MTI946	*Klebsiella pneumoniae* ATCC 13883	1	1	32	0.5	16	0.0625/1.2	0.0625/1.2	RA	RA	RA	32	16	RA	RA	RA	4	1	RA	RA	RA	2	8	RA	RA	RA
	MTI947	*Klebsiella pneumoniae* ATCC 13883	2	1	32	4	128	0.25/4.8	0.00125/0.6	RA	RA	RA	16	8	RA	RA	RA	2	1	RA	RA	RA	1	4	RA	RA	RA
	Clinical Breakpoints:																										
	MTI945	*Pseudomonas aeruginosa* ATCC 10145	512	>512	>512	16	256	32/608	32/608	RA	RA	RA	>512	512	RA	RA	RA	64	32	RA	RA	RA	64	512	RA	RA	RA

			Neomycin (µg/mL)					Gentamicin (µg/mL)					Tetracycline (µg/mL)					Azithromycin (µg/mL)					Erythromycin (µg/mL)				
	Clinical Breakpoints:		S ≤ 8, I = 16, R ≥ 32[171]					S ≤ 4, I = 8, R ≥ 16					S ≤ 4, I = 8, R ≥ 16					S ≤ 16, R ≥ 32[107]									
	Strain	Strain Name	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Enterobacteriaceae	MTI942	*Shigella flexneri* ATCC 29903	4	4	RA	RA	RA	1	2	RA	RA	RA	1	4	RA	RA	RA	2	1	RA	RA	RA	32	16	RA	RA	RA
	MTI944	*Providencia stuartii* ATCC 29914	0.5	8	RA	RA	RA	0.5	2	RA	RA	RA	128	64	RA	RA	RA	32	16	RA	RA	RA	256	128	RA	RA	RA
	MTI946	*Klebsiella pneumoniae* ATCC 13883	1	4	RA	RA	RA	0.5	1	RA	RA	RA	1	8	2	0.5	1	8	8	RA	RA	RA	256	128	RA	RA	RA
	MTI947	*Klebsiella pneumoniae* ATCC 13883	0.5	2	RA	RA	RA	0.25	0.5	RA	RA	RA	1	16	2	1	4	8	4	RA	RA	RA	64	64	RA	RA	RA
	Clinical Breakpoints:							S ≤ 4, I = 8, R ≥ 16																			
	MTI945	*Pseudomonas aeruginosa* ATCC 10145	16	256	RA	RA	RA	4	32	RA	RA	RA	64	>512	32	1	4	256	32	RA	RA	RA	512	128	RA	RA	RA

			Chloramphenicol (µg/mL)					Florfenicol (µg/mL)					Nalidixic Acid (µg/mL)					Ciprofloxacin (µg/mL)					Enrofloxacin (µg/mL)				
	Clinical Breakpoints:		S ≤ 8, I = 16, R ≥ 32										S ≤ 16, R ≥ 32					S ≤ 1, I = 2, R ≥ 4					S ≤ 0.5; I = 1; R ≥ 2[169]				
	Strain	Strain Name	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5	CaMHB	DMEM	MHB pH 5.5	LPM pH 7	LPM pH 5.5
Enterobacteriaceae	MTI942	*Shigella flexneri* ATCC 29903	2	1	RA	RA	RA	1	1	RA	RA	RA	2	8	RA	RA	RA	0.0195	0.0078	RA	RA	RA	0.0195	0.0625	RA	RA	RA
	MTI944	*Providencia stuartii* ATCC 29914	32	32	RA	RA	RA	4	8	RA	RA	RA	2	4	RA	RA	RA	0.0195	0.0195	RA	RA	RA	0.0625	0.125	RA	RA	RA
	MTI946	*Klebsiella pneumoniae* ATCC 13883	4	8	8	4	8	4	8	8	8	8	4	8	2	1	2	0.0039	0.0039	RA	RA	RA	0.0195	0.00125	RA	RA	RA
	MTI947	*Klebsiella pneumoniae* ATCC 13883	8	8	8	8	16	4	8	8	8	16	4	16	4	16	8	0.00125	0.00125	RA	RA	RA	0.00125	0.125	RA	RA	RA
	Clinical Breakpoints:																	S ≤ 1, I = 2, R ≥ 4									
	MTI945	*Pseudomonas aeruginosa* ATCC 10145	256	128	256	4	16	512	256	256	8	64	256	256	64	16	256	0.125	0.25	RA	RA	RA	1	2	RA	RA	RA

MHB and DMEM MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO_2 incubator. S = Susceptible, IRS = Non-Susceptible, I = Intermediate, R = Resistant

[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[36] unless otherwise indicated

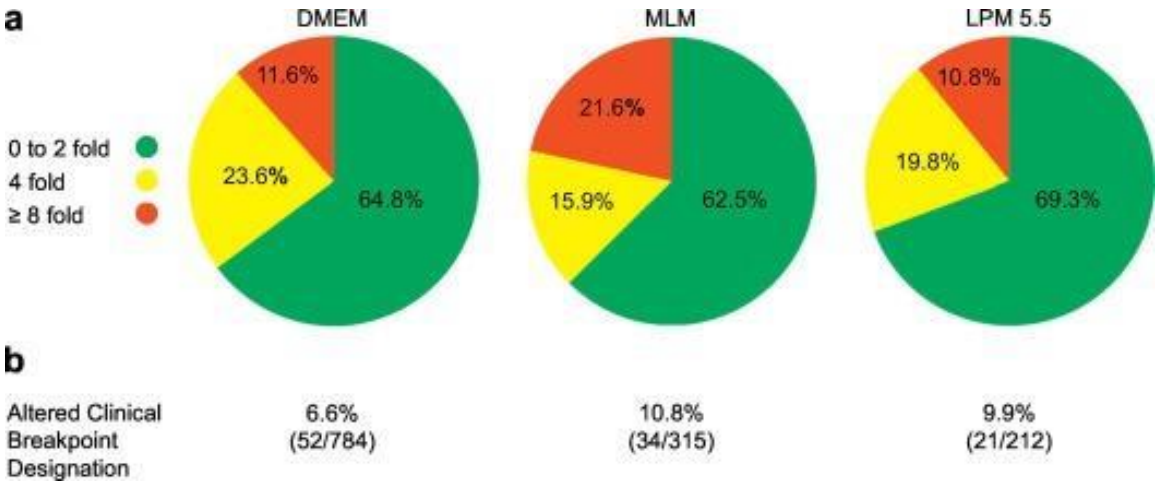

Figure 2. Comparison summary of MICs derived from host-mimicking media versus standard MHB medium. (a) Colored regions depict the fraction of pathogen-antibiotic combinations tested that exhibited a fold-change in MICs (increased susceptibility or resistance) when derived in host-mimicking media (DMEM, MLM, LPM pH 5.5) relative to standard MHB medium (test/standard condition); ≤2-fold (green), 4-fold (yellow), ≥8-fold (red). (b) Depicted are percentages of pathogen-antibiotic combinations that resulted in altered MICs that crossed clinical breakpoint designations, used to define isolates as susceptible ("S"), intermediate ("I"), or resistant ("R"), that can impact clinical decision making on appropriate antibiotic therapy (Clinical and Laboratory Standards Institute, 2012a; European Committee on Antibiotic Susceptibility Testing, 2014).

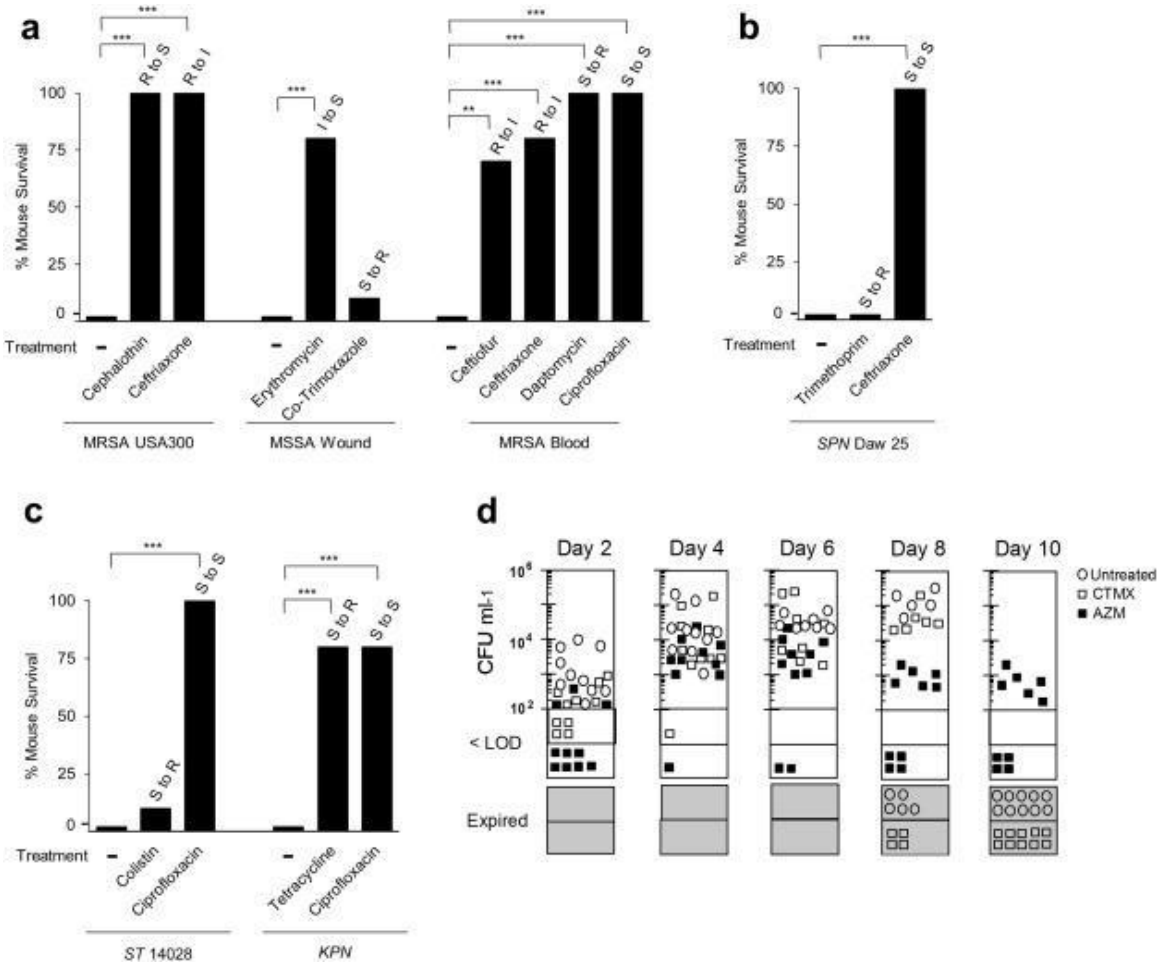

Figure 3. Antibiotic susceptibility testing in host-mimicking media improves the predictive value of AST in the assignment of appropriate antibiotic therapy in murine models of sepsis. Pathogen-antibiotic combinations that exhibited altered MICs in host-mimicking media relative to standard MHB medium and whose MICs crossed clinical breakpoint designations were evaluated in murine sepsis models of (a) *S. aureus* (MRSA [USA300]; MSSA Wound [MT3307]; MRSA Blood [MT3302]); (b*) S. pneumoniae* (*SPN* Daw25); and (c) *S*. Typhimurium (*ST* 14028) and *K. pneumoniae* (KPN ATCC13883). (d) MSSA (MT3307) clearance from blood circulation was examined following treatment with antibiotics predicted as highly effective via testing in standard MHB medium (co-trimoxazole, open boxes) or tissue culture medium (DMEM) (azithromycin, closed boxes), respectively. Untreated mice (open circles); expired mice (gray region); Colony Forming Units (CFU); Limit of Detection (LOD) = 100 CFU/ml (Patterson et al., 2013). Ten mice were evaluated per cohort. ***$P < 0.001$, **$P < 0.01$, or *$P < 0.05$.

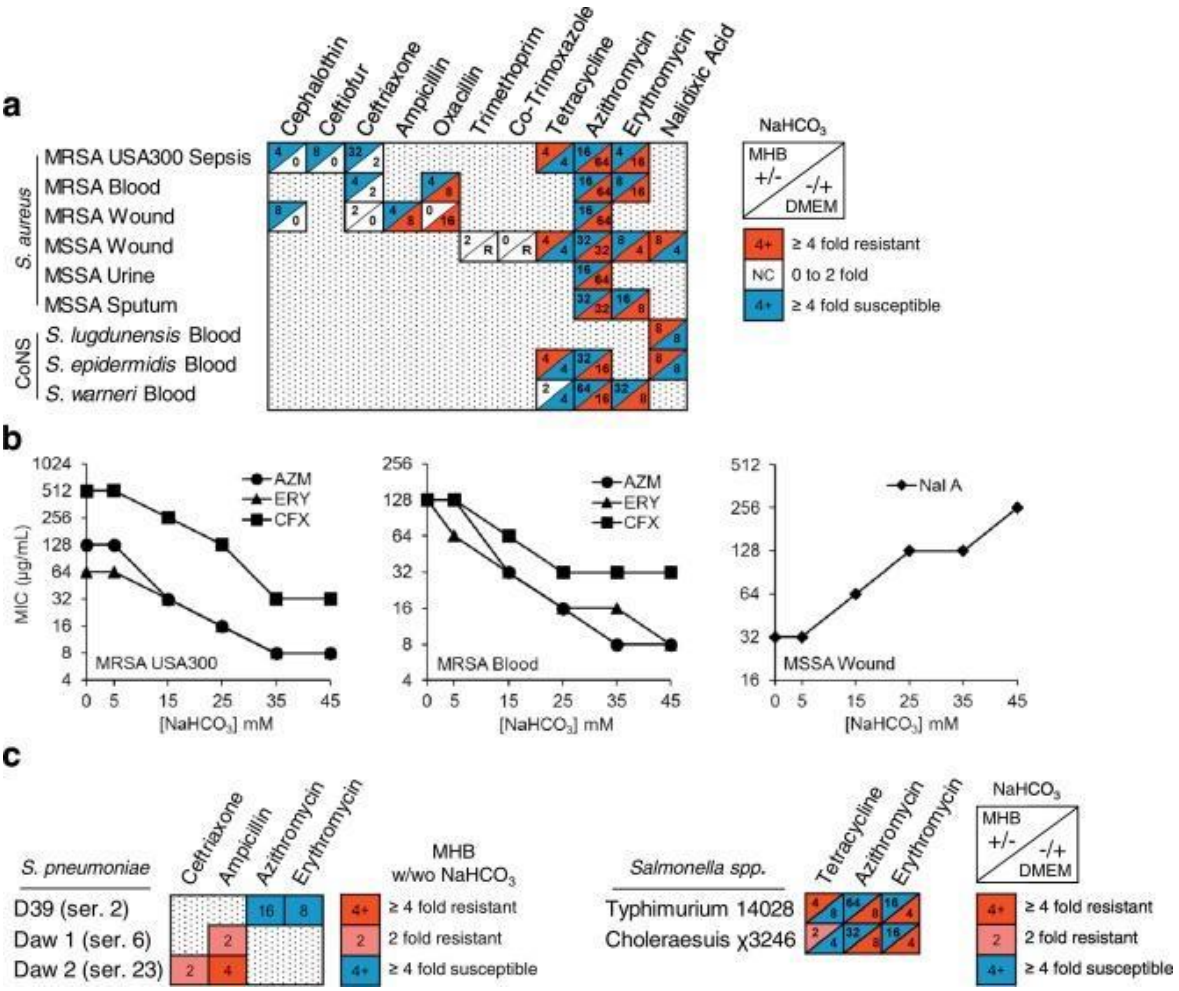

Figure 4. Supplementation of standard MHB medium with physiological levels of $NaHCO_3$ improves the predictive value of AST in the assignment of appropriate antibiotics for therapeutic intervention. (a) *S. aureus* exhibiting at least an 8-fold change in MIC in tissue culture medium (DMEM) vs. MHB medium were subjected to susceptibility tests in the presence and absence of physiological levels of $NaHCO_3$. Values represent MIC fold-change when derived in MHB medium in the presence/absence of $NaHCO_3$ (test/standard condition; left of slash); and in DMEM medium in the absence/presence of $NaHCO_3$ (test/standard condition; right of slash). Increased susceptibility is depicted in blue; increased resistance is depicted in red. Stippled boxes represent those that exhibited <8-fold altered susceptibility between MHB and DMEM media. To control for pH and buffer considerations, strains were grown in MHB pH 7.2; MHB adjusted to pH 7.2 w/100 mM Tris; and DMEM liquid pH 7.4 (containing 44 mM $NaHCO_3$); all other media conditions were adjusted to pH 7.4 with 100 mM Tris including: MHB w/$NaHCO_3$; and $NaHCO_3$-free powdered DMEM w/wo $NaHCO_3$ (**Table 3a**). (b) Dose response analysis of MRSA (USA300; MT3302) and MSSA (MT3307) antibiotic susceptibility following exposure to increasing concentrations of $NaHCO_3$ in standard MHB medium. AZM (azithromycin); ERY (erythromycin); CFX (ceftriaxone). (c) Susceptibility of *S. pneumoniae* and *Salmonella* spp. in the presence/absence of

physiological levels of $NaHCO_3$ in MHB and/or DMEM media. For *S. pneumoniae*, values represent fold-change between MICs derived in MHB medium in the presence/absence of $NaHCO_3$ (test/standard condition). For *Salmonella* spp. values represent fold-change between MICs derived in MHB medium in the presence/absence of $NaHCO_3$ (test/standard condition; left of slash); and DMEM in the absence/presence of $NaHCO_3$ (test/standard condition; right of slash). No change (NC), Resistant (R). MICs were a consensus of at least 6 independent isolates.

Table 3a. Antimicrobial susceptibility test in media w/ and w/o $NaHCO_3$ (*Staphylococcus*)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

		Cephalothin MIC (µg/mL)					
Clinical Breakpoints*:		S ≤ 8; I = 16; R ≥ 32[2]					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	32	32	8	4	4	4
MT3316	MRSA Wound	8	4	1	0.5	0.5	0.5

		Ceftiofur MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 2; I = 4; R ≥ 8[3]					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	64	128	8	8	4	8

		Ceftriaxone MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 8; I = 16-32; R ≥ 64[2]					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	512	256	16	16	16	32
MT3302	MRSA Blood	128	64	32	16	8	8
MT3316	MRSA Wound	64	64	32	8	16	8

		Ampicillin MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 0.25; R ≥ 0.5[2]					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3316	MRSA Wound	8	8	2	0.5	1	4

		Oxacillin MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 2; R ≥ 4					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3302	MRSA Blood	64	64	16	4	8	32
MT3316	MRSA Wound	32	64	32	1	1	16

		Trimethoprim MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 8; R ≥ 16					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3307	MSSA Wound	2	1	1	>512	>512	>512

		Co-Trimoxazole MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 2/38; R ≥ 4/76					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3307	MSSA Wound	0.125/2.4	0.0625/1.2	0.125/2.4	>64/1216	>64/1216	>64/1216

		Tetracycline MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 4; I = 8; R ≥ 16					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	0.5	0.5	2	4	2	1
MT3307	MSSA Wound	0.5	0.5	2	4	2	1
MT3320	CoN *S. epidermidis*	0.5	0.25	2	4	2	1
MT3321	CoN *S. warneri*	0.5	0.25	1	4	2	1

		Azithromycin MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 2; I = 4; R ≥ 8					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	128	128	8	8	16	512
MT3302	MRSA Blood	128	256	8	8	16	512
MT3316	MRSA Wound	1	2	0.0625	0.125	0.25	8
MT3307	MSSA Wound	2	2	0.0625	0.125	0.25	4
MT3309	MSSA Urine	1	2	0.0625	0.125	0.25	8
MT3314	MSSA Sputum	2	2	0.0625	0.125	0.25	4
MT3320	CoN *S. epidermidis*	256	256	8	32	64	512
MT3321	CoN *S. warneri*	256	128	4	32	64	512

		Erythromycin MIC (µg/mL)					
Clinical Breakpoints:		S ≤ 0.5; I = 1 - 4; R ≥ 8					
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3322	MRSA USA300	64	64	16	8	8	128
MT3302	MRSA Blood	128	128	16	8	16	128
MT3307	MSSA Wound	1	0.5	0.125	0.125	0.25	0.5
MT3314	MSSA Sputum	1	0.5	0.0625	0.125	0.25	1
MT3321	CoN *S. warneri*	256	128	8	32	64	256

		Nalidixic Acid MIC (µg/mL)					
Clinical Breakpoints:							
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4	DMEM + 5% LB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM + 5% LB 100 mM Tris (w/o $NaHCO_3$) pH 7.4
MT3307	MSSA Wound	32	32	256	256	256	64
MT3317	CoN *S. lugdunensis*	64	128	512	512	512	64
MT3320	CoN *S. epidermidis*	32	64	256	256	128	32

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5 % CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

*All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated.

Table 3b. Antimicrobial susceptibility test in media w/ and w/o $NaHCO_3$ (*Streptococcus*)

		Ceftriaxone MIC (µg/mL)		
Clinical Breakpoints[a]:		S ≤ 1; I = 2; R ≥ 4		
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB 100 mM Tris pH 7.2	Ca-MHB + 5% LHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4
Daw 2	23	2	2	4

		Ampicillin MIC (µg/mL)		
Clinical Breakpoints:		S ≤ 0.5; I = 1-2; R ≥ 4[6]		
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB 100 mM Tris pH 7.2	Ca-MHB + 5% LHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4
Daw 1	6	2	2	4
Daw 2	23	0.5	1	2

		Azithromycin MIC (µg/mL)		
Clinical Breakpoints:		S ≤ 0.5; I = 1; R ≥ 2		
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB 100 mM Tris pH 7.2	Ca-MHB + 5% LHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4
D39	2	0.25	0.25	0.0156

		Erythromycin MIC (µg/mL)		
Clinical Breakpoints:		S ≤ 0.25; I = 0.5; R ≥ 1		
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB 100 mM Tris pH 7.2	Ca-MHB + 5% LHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4
D39	2	0.125	0.125	0.0156

All MICs were determined by broth microdilution in accordance with CLSI guidelines. MIC plates were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated.

Table 3c. Antimicrobial susceptibility test in media w/ and w/o $NaHCO_3$ (*Salmonella*)

	Tetracycline MIC (µg/mL)					
Clinical Breakpoints[a]:	S ≤ 4, I = 8, R ≥ 16					
Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM pH 7.4	DMEM 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM 100 mM Tris (w/o $NaHCO_3$) pH 7.4
S. Typhimurium 14028	1	1	4	8	8	1
S. Choleraesuis χ3246	1	1	2	8	4	2

	Azithromycin MIC (µg/mL)					
Clinical Breakpoints:	S ≤ 16, R ≥ 32[6]					
Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM pH 7.4	DMEM 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM 100 mM Tris (w/o $NaHCO_3$) pH 7.4
S. Typhimurium 14028	8	8	0.125	2	2	16
S. Choleraesuis χ3246	8	8	0.25	2	2	16

	Erythromycin MIC (µg/mL)					
Clinical Breakpoints:						
Strain Name	Ca-MHB pH 7.2	Ca-MHB 100 mM Tris pH 7.2	Ca-MHB 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM pH 7.4	DMEM 100 mM Tris 44 mM $NaHCO_3$ pH 7.4	DMEM 100 mM Tris (w/o $NaHCO_3$) pH 7.4
S. Typhimurium 14028	128	128	8	32	32	128
S. Choleraesuis χ3246	128	64	8	32	32	128

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a]All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated.

Table 2 & Table 3 References

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Chapter 3: Supplementing test media with bicarbonate or fetal bovine serum results in changes to antimicrobial susceptibility

3.1 Introduction

Bacteria have developed complex signal transduction systems which enable them to alter gene expression in response to various environmental cues. Sodium bicarbonate ($NaHCO_3$) is an abundant ionic factor found in mammalian tissues that stimulates changes in bacterial structure, membrane permeability, and/or gene expression (Dorschner et al., 2006). Sodium bicarbonate-induced global changes to bacteria resulted in changes in susceptibility to antimicrobial peptides, as shown by altered AST results when bicarbonate was added to standard MHB or removed from tissue culture medium (Ersoy et al., 2017). Sodium bicarbonate further proved to be a host-mimicking signal for a wide variety of strains for several drugs, as shown by the fact that susceptibility profiles obtained from $NaHCO_3$-supplemented media more accurately predicted the *in vivo* response than those obtained with the standard MHB (Ersoy et al., 2017). Here, we screened all other strains for altered resistance to antibiotics when the standard MHB testing medium was supplemented with sodium bicarbonate.

In addition to sodium bicarbonate, components of fetal bovine serum (FBS) play a role in signal transduction in many cell types, whether it be desensitizing a receptor, inducing protein translocation, or causing cytokine release in macrophages during bacterial infection (Berenguer, Martinez, Giorgetti-Peraldi, Le Marchand-Brustel, & Govers, 2010; Brar et al., 1999; Chrisman, Perkins, & Garbers, 2003; Flesch & Kaufmann, 1999; Wang, Maier, Wenz, Giordano, & Herskind, 2013). Since serum factors have been shown to be functionally involved in many

processes, we had reason to believe that the addition of FBS to host-mimicking media for AST testing would lead to changes in a strain's resistance or susceptibility to a drug. We screened a subset of drugs for 10 *Staphylococcus* strains and 5 *E. coli* strains, using tissue culture medium, Dulbecco's Modified Eagle Medium (DMEM), supplemented with various levels of FBS as the testing media.

3.2 Results

3.2.1 Addition of $NaHCO_3$ to standard MHB medium affects antimicrobial susceptibility

We completed MIC testing in MHB supplemented with $NaHCO_3$, for all strain and drug combinations that had not been previously determined as described in Ersoy et al. (2017; chapter 2). These included *Staphylococcus* spp., *Streptococcus pneumoniae,* and Gram-negative strains. Four categories were identified which describe the possible breakpoint changes resulting from addition of $NaHCO_3$ to the standard MHB (**Table 4**): 1) MICs performed in MHB vs DMEM have different breakpoints and adding $NaHCO_3$ to MHB results in the same breakpoint as DMEM; 2) MICs performed in MHB and DMEM have the same breakpoint and adding $NaHCO_3$ to MHB changes this breakpoint; 3) MICs performed in MHB vs DMEM have different breakpoints and adding $NaHCO_3$ to MHB results in the same breakpoint as MHB; and 4) MICs performed in MHB and DMEM have the same breakpoint and adding $NaHCO_3$ to MHB does not change this breakpoint. In categories 1 and 2, MHB supplemented with $NaHCO_3$ yields differing breakpoints than the standard MHB used for AST testing, indicating an effect of sodium bicarbonate on antimicrobial susceptibility. Interestingly, category 1 findings

demonstrate the possibility of obtaining the same breakpoint designations obtained by host-mimicking media by simply adding a signal-inducing molecule to the standard MHB media. In categories 3 and 4, sodium bicarbonate did not change the breakpoint when added to standard MHB.

Staphylococcus

Staphylococcus strains grown in MHB supplemented with $NaHCO_3$ (MHB+$NaHCO_3$) demonstrated trends of increased susceptibility to azithromycin and erythromycin and increased resistance to nalidixic acid (**Fig. 5a**, (Ersoy et al., 2017)). However, these large fold-changes in MIC values between MHB and MHB+$NaHCO_3$ did not cross clinical breakpoints (**Table 5a**).

Category 1 consists of instances where supplementing standard MHB with $NaHCO_3$ resulted in a breakpoint that resembled the DMEM breakpoint and differed from the MHB breakpoint. For example, MRSA USA300 and cephalothin demonstrated a shift from the “R” breakpoint in MHB to the “S” breakpoint seen in both MHB+$NaHCO_3$ and DMEM (**Table 1**), categorizing this drug and strain combination as Category 1. New findings include clinical breakpoint changes showing increased susceptibility of MSSA Urine (R to S) and MSSA Sputum (R to I) to streptomycin, as well as increased resistance of MSSA Urine (S to I) to tetracycline, when $NaHCO_3$ was added to MHB (**Table 4a**, **Table 5a**).

Category 2 portrays instances where addition of $NaHCO_3$ to MHB results in a clinical breakpoint that is different than the breakpoint shared by MHB and DMEM. For example, MSSA Blood, MSSA Wound, and MSSA Urine are “Susceptible” to ceftiofur in MHB and DMEM, but “Intermediate” to ceftiofur in MHB+$NaHCO_3$ (**Table**

4a, **Table 5a**). Even more significant is the shift in clinical breakpoints seen in MSSA Wound and MSSA Urine, which are “Susceptible” to ampicillin in MHB and DMEM, but “Resistant” to ampicillin when MHB is supplemented with $NaHCO_3$. Lastly, MSSA Urine shows increased resistance to ceftriaxone (S to I) with the addition of $NaHCO_3$ to MHB.

Streptococcus pneumoniae

All 6 *Streptococcus pneumoniae* strains grown in MHB + $NaHCO_3$ showed significantly increased susceptibility (16- or 32-fold) to azithromycin compared to those grown in MHB ((Ersoy et al., 2017), **Fig. 5b**), but these large fold changes did not cross clinical breakpoints (**Table 5b**). Conversely, a small 2-fold change in MIC values between MHB and MHB+$NaHCO_3$ resulted in breakpoint changes for Daw 25 (R to S, trimethoprim) and Daw 2 (R to I, co-trimoxazole). Additionally, Daw 1 was “Susceptible” to ceftriaxone in MHB and DMEM, but “Intermediate” to ceftriaxone in MHB+$NaHCO_3$, with a 4-fold change in MIC value between MHB and MHB+$NaHCO_3$.

Gram-negative Bacteria

Trends of increased susceptibility (8- to 64-fold) to azithromycin and erythromycin were seen among *Salmonella* and *Escherichia coli (E. coli)* strains when $NaHCO_3$ was added to MHB (**Fig. 5c**). Increased susceptibility (8- or 16-fold) to azithromycin in MHB+$NaHCO_3$ was also found for *Providencia stuartii*, *Citrobacter freundii*, and *Pseudomonas aeruginosa*. *Providencia stuartii* changed

breakpoints from “R” in MHB to “S” in MHB+$NaHCO_3$ when tested with azithromycin (**Table 4c**).

Additionally, trends of increased resistance (4- or 8-fold) were seen among *E. coli* strains to ampicillin, nalidixic acid, and enrofloxacin (**Fig. 5c**). While no changes in breakpoint between MHB and MHB+$NaHCO_3$ were found with nalidixic acid, breakpoint changes were observed for ampicillin and enrofloxacin. *Citrobacter freundii* (I to R) and *E. coli* strains ATCC 25922 (S to I), UPEC J96 (S to R), UPEC ECR12 (S to I), UPEC ATCC 11775 (S to I), APEC χ7126 (S to I), A96 χ7117 (S to R), EPEC χ2927 (S to R), and RDEC-1 χ2862 (S to R) all showed increased resistance to ampicillin when $NaHCO_3$ was added to MHB. Similarly, *E. coli* strains EPEC χ2927, UPEC J96, UPEC ECR12, and EPEC JPN 15 all indicated increased resistance to enrofloxacin as shown by an “S” to “I” change in clinical breakpoints with $NaHCO_3$ addition to MHB.

3/4 *Yersinia* strains tested showed an 8- or 16-fold increase in resistance to tetracycline, and these changes crossed clinical breakpoints (S to R; S to I) (**Table 4c**, **Table 5c**). Additionally, the *Salmonella* strain S. Dublin displayed an “R” breakpoint in MHB and an “S” breakpoint in MHB+$NaHCO_3$ when tested with Polymyxin B (16-fold MIC change) and Colistin Sulfate (8-fold MIC change).

3.2.2 Addition of fetal bovine serum (FBS) to tissue culture medium affects antimicrobial susceptibility

To further test for possible changes in antimicrobial susceptibility resulting from the presence of extracellular signals, we investigated the effects of fetal bovine serum (FBS) on AST results for *Staphylococcus* strains and Gram-negative strains

in response to a selection of drugs. Since FBS is known to induce a variety of signals in different cell types, we supplemented tissue culture medium, Dulbecco's Modified Eagle Medium (DMEM), with increasing concentrations of fetal bovine serum (FBS) to test for changes in antimicrobial susceptibility compared to DMEM alone or MHB alone.

Staphylococcus

7/10 *Staphylococcus* strains grown in DMEM supplemented with FBS (DMEM + FBS) show increased resistance to ceftiofur compared to DMEM alone or MHB alone (**Fig. 6a**). MSSA Blood, MSSA Wound, MSSA Urine, MSSA Sputum, CoN *S. lugdunensis*, CoN *S. epidermidis*, and CoN *S. warneri* show increased resistance to ceftiofur in a dose-dependent manner, as shown by the gradual increases in resistance as the percentage of FBS in DMEM increases (**Table 6a**). Further, these 7 *Staphylococcus* strains undergo changes in clinical breakpoints with the addition of FBS to DMEM. For example, MSSA Blood is "Susceptible" to ceftiofur in DMEM with 0% FBS, "Intermediate" in DMEM supplemented with either 5% or 10% FBS, and "Resistant" to ceftiofur in DMEM supplemented with either 20% or 50% FBS. Additionally, CoN *S. warneri* is "Susceptible" to ceftiofur in DMEM supplemented with 0%, 5%, 10%, or 20% FBS, and "Intermediate" in DMEM supplemented with 50% FBS.

Dose-dependent changes in MIC values and clinical breakpoints in response to FBS is also seen with MSSA Sputum and chloramphenicol (**Table 6a**). MSSA Sputum is "Susceptible" to chloramphenicol in DMEM with 0%, 5%, and 10% FBS, "Intermediate" in DMEM with 20% FBS, and "Resistant" to chloramphenicol in

DMEM with 50% FBS. Various other *Staphylococcus* strains (MRSA USA300, MRSA Wound, MSSA Blood, MSSA Wound, MSSA Urine, CoN *S. epidermidis*, and CoN *S. warneri*) also seem to respond to the increased FBS in DMEM as shown by 2-fold increases in resistance to chloramphenicol that cross clinical breakpoints (S to I; I to R).

Gram-negative bacteria

2/5 Gram-negative strains tested (*S*. Typhimurium 14028, *E. coli* strain APEC χ7126) showed 4-fold increases in resistance to nalidixic acid from DMEM + 0% FBS to DMEM + 50% FBS (**Fig. 6b**). For *S*. Typhimurium 14028, this increase in resistance to nalidixic acid crossed a clinical breakpoint as the strain is "Susceptible" in DMEM with 0%, 5%, 10%, and 20% FBS, but "Resistant" in DMEM with 50% FBS (**Table 6b**).

There are no defined clinical breakpoints for *S*. Typhimurium 14028 in erythromycin, but the strain undergoes a seemingly dose-dependent increase in resistance, as shown by the MIC value of 32µg/mL in DMEM with 0% FBS, 64µg/mL in DMEM with 5% and 10% FBS, and 128µg/mL in DMEM with 20% and 50% FBS (**Table 6b**). Additionally, *S*. Typhimurium TY1212 undergoes a 4-fold increase in susceptibility to ceftiofur from DMEM with 0% FBS to DMEM with 5%, 10%, 20%, and 50% FBS, without crossing any breakpoints.

While only a 2-fold change in the MIC value, *S.* Typhimurium 14028 crosses a breakpoint (S to I) such that the strain is "Susceptible" to spectinomycin in DMEM with 0%, 5%, and 10% FBS, but "Intermediate" in DMEM with 20% and 50% FBS. Similarly, *E. coli* strain APEC χ7126 undergoes a 2-fold increase in resistance to

chloramphenicol, changing the breakpoint from “Susceptible” in DMEM with 0%, 5%, and 10% FBS to “Intermediate” in DMEM with 20% and 50% FBS. Lastly, a 2-fold increase in resistance of *S*. Typhimurium TY1212 to nalidixic acid shifts the “Susceptible” breakpoint in DMEM with 0%, 5%, and 10% FBS to “Resistant” in DMEM with 20% and 50% FBS.

3.3 Discussion

Overall, there are several instances of changes in clinical breakpoints resulting from addition of either $NaHCO_3$ or FBS to media. These changes in clinical breakpoints indicate that caution should be taken when prescribing the antibiotic for that infection. Further, these changes reveal the risk of testing in a single media because crucial information that may change the treatment decision will be missed. If testing in multiple media was implemented in clinical settings, it would enable multiple susceptibility profiles to be gathered and compared, whereas the current standard AST testing only provides a single MIC value and breakpoint designation. Further, when clinical breakpoint changes are observed between two testing media, additional signal-inducing media should be included in the analysis of the strain’s susceptibility to that drug. Additionally, *in vivo* mouse experiments should be conducted to investigate the accuracy of the AST results presented in this thesis and the ability of the “host-mimicking” media to truly represent the host environment. Although *in vivo* experiments would not be able to follow the often rushed timeline of clinical testing, they have been shown to support the susceptibility profiles obtained when AST was performed in host-mimicking media.

There are also several cases of large fold changes between MIC values obtained from the standard media versus the alternative media, but they do not cross clinical breakpoints. However, since these instances are clearly affected by extracellular signals, they should be further investigated in different host-mimicking media. On the other hand, there are cases where a small 2-fold change in MIC values crosses a clinical breakpoint. Two-fold differences in MIC values are generally considered to be insignificant. However, in these cases, we should be aware that the MIC value is on the cusp of its clinical breakpoint designation and thus a simple change in testing media may hold a greater weight on its AST susceptibility profile.

It is also important to recognize that in some cases the gradual changes in MIC values between 0%-50% FBS seem to be dose-dependent. In these cases especially, testing with an increased percentage of FBS (50-100%) may further increase the 2-fold change and possibly even change the clinical breakpoint. These findings imply that testing with greater percentages of FBS may lead to greater changes in MIC values and possibly changes in breakpoints as well.

While not every drug and strain combination demonstrates a breakpoint change or significant fold-change between standard MHB and alternative media, it has been made clear that AST results often vary – and possibly even improve – with the presence of signaling molecules in the testing media. Current AST testing does not allow for these discoveries as only one standard media is used to make a decision on the antibiotic to prescribe. A greater variety of testing media that can be used for

AST testing would enable more data and more informed decisions on the best drug to treat infections with.

Figures

Table 4. Adding $NaHCO_3$ to MHB media results in MICs that cross clinical breakpoint designations which advise on patient therapy. Depicted are instances of clinical breakpoint changes resulting from the addition of 44mM $NaHCO_3$ to standard MHB, for (a) *Staphylococcus* spp., (b) *Streptococcus pneumoniae*, and (c) Gram-negative bacteria.

a)

Addition of $NaHCO_3$ to MHB has an effect on MIC breakpoint			
1. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in the DMEM breakpoint		Breakpoint change (MHB → MHB Tris $NaHCO_3$/DMEM)	Fold change (MHB → MHB Tris $NaHCO_3$)
Cephalothin	MRSA USA300 *	R → S	4
Ceftriaxone	MRSA USA300 *	R → I	32
	MRSA Blood *	R → I	4
	MRSA Wound *	R → I (MHB Tris $NaHCO_3$) / S (DMEM)	2
Streptomycin	MSSA Urine	R → S	4
	MSSA Sputum	R → I (MHB Tris $NaHCO_3$) / S (DMEM)	2
Tetracycline	MSSA Urine	S → I	2
Erythromycin	MSSA Wound *	I → S	8
	MSSA Sputum *	I → S	16
2. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB changes this breakpoint		Breakpoint change (MHB/DMEM → MHB Tris $NaHCO_3$)	Fold change (MHB → MHB Tris $NaHCO_3$)
Ceftiofur	MSSA Blood	S → I	4
	MSSA Wound	S → I	4
	MSSA Urine	S → I	4
Ceftriaxone	MSSA Urine	S → I	4
Ampicillin	MSSA Wound	S → R	4
	MSSA Urine	S → R	4
Azithromycin	CoN S. warneri *	R → I	64

Addition of $NaHCO_3$ to MHB has no effect on MIC breakpoint		
3. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in MHB breakpoint		Breakpoint change (MHB/MHB Tris $NaHCO_3$ → DMEM)
Ceftiofur	MRSA Blood	R → I
	MRSA Wound	R → I
Oxacillin	MRSA Wound *	R → S
Trimethoprim	MSSA Wound *	S → R
Co-Trimoxazole	MSSA Wound *	S → R
Chloramphenicol	MSSA Wound	S → I
4. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB does not change this breakpoint		
All other drug & strains combinations not listed above		

* Previously published findings

b)

Addition of $NaHCO_3$ to MHB has an effect on MIC breakpoint			
1. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in the DMEM breakpoint		Breakpoint change (MHB → MHB Tris $NaHCO_3$/DMEM)	Fold change (MHB → MHB Tris $NaHCO_3$)
Ceftriaxone	Daw 2, serotype 23 *	I → R	2
Ampicillin	Daw 1, serotype 6 *	I → R (MHB Tris $NaHCO_3$) / S (DMEM)	2
	Daw 2, serotype 23 *	S → I (MHB Tris $NaHCO_3$) / R (DMEM)	4
Trimethoprim	Daw 25, serotype 35C	R → S	2
Co-Trimoxazole	Daw 2, serotype 23	R → I	2
2. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB changes this breakpoint		Breakpoint change (MHB/DMEM → MHB Tris $NaHCO_3$)	Fold change (MHB → MHB Tris $NaHCO_3$)
Ceftriaxone	Daw 1, serotype 6	S → I	4

Addition of $NaHCO_3$ to MHB has no effect on MIC breakpoint		
3. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in MHB breakpoint		Breakpoint change (MHB/MHB Tris $NaHCO_3$ → DMEM)
Ampicillin	Daw 19, serotype 6	R → S
Trimethoprim	Daw 1, serotype 6	R → S
Co-Trimoxazole	Daw 1, serotype 6	R → I
4. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB does not change this breakpoint		
All other drug & strains combinations not listed above		

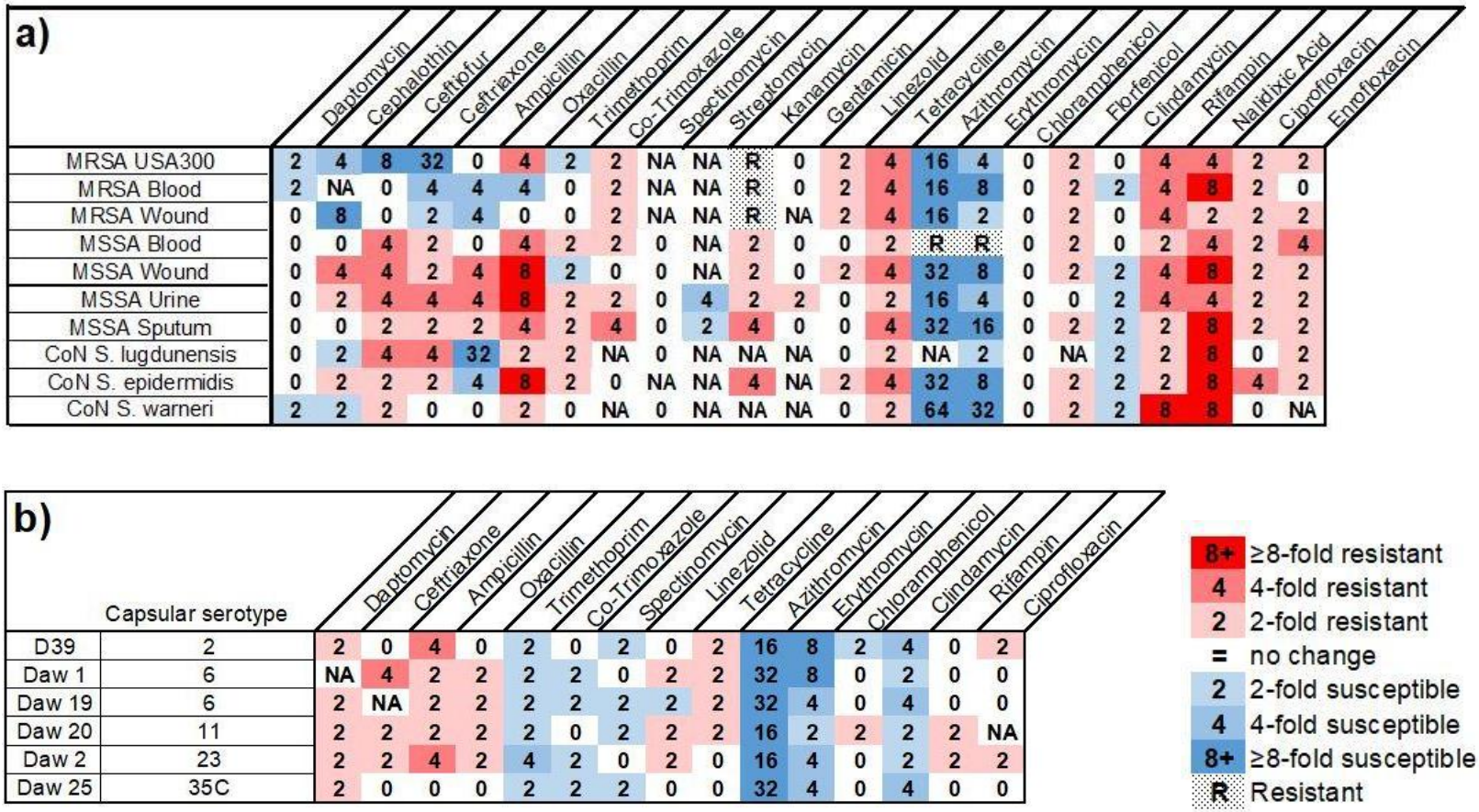
* Previously published findings

c)

Addition of $NaHCO_3$ to MHB has an effect on MIC breakpoint			
1. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in the DMEM breakpoint		Breakpoint change (MHB → MHB Tris $NaHCO_3$/DMEM)	Fold change (MHB → MHB Tris $NaHCO_3$)
Polymyxin B	S. Dublin Lane	R → S	16
Ampicillin	*Citrobacter freundii* ATCC 8090	I → R	4
Spectinomycin	*Shigella flexneri* ATCC 29903	I → S	2
Tetracycline	RDEC-1 χ2862	S → I	4
	Y. pseudotuberculosis IP32953	S → R (MHB Tris $NaHCO_3$) / I (DMEM)	16
	Y. pseudotuberculosis IP2515	S → I	8
	Y. pseudotuberculosis IP2666	S → I	8
Azithromycin	*Providencia stuartii* ATCC 29914	R → S	8
Florfenicol	S. Typhimurium 14028	I → S	2
Enrofloxacin	EPEC χ2927	S → I (MHB Tris $NaHCO_3$) / R (DMEM)	4
2. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB changes this breakpoint		Breakpoint change (MHB/DMEM → MHB Tris $NaHCO_3$)	Fold change (MHB --> MHB Tris $NaHCO_3$)
Polymyxin B	*Y. pseudotuberculosis* IP32953	R → S	256
Colistin Sulfate	S. Dublin Lane	R → S	8
Ampicillin	Seattle 1946; O6 biotype 1 ATCC 25922	S → I	4
	UPEC J96	S → R	8
	UPEC ECR12	S → I	4
	UPEC ATCC 11775	S → I	4
	APEC χ7126	S → I	8
	A96 χ7117	S → R	8
	EPEC χ2927	S → R	8
	RDEC-1 χ2862	S → R	8
Chloramphenicol	*Providencia stuartii* ATCC 29914	R → I	2
Enrofloxacin	UPEC J96	S → I	4
	UPEC ECR12	S → I	8
	EPEC JPN 15	S → I	4

Addition of $NaHCO_3$ to MHB has no effect on MIC breakpoint		
3. MHB & DMEM have different breakpoints & adding $NaHCO_3$ to MHB results in MHB breakpoint		Breakpoint change (MHB/MHB Tris $NaHCO_3$ → DMEM)
Polymyxin B	*Pseudomonas aeruginosa* ATCC 10145	S → R
Colistin Sulfate	S. Typhimurium 14028	S → R
	Pseudomonas aeruginosa ATCC 10145	S → R
Streptomycin	S. Dublin Lane	R → S
Gentamicin	*Pseudomonas aeruginosa* ATCC 10145	S → R
Tetracycline	S. Typhimurium 14028 *	S → I
	S. Choleraesuis χ3246 *	S → I
	Seattle 1946; O6 biotype 1 ATCC 25922	S → I
	UPEC J96	S → I
	UPEC ECR12	S → I
	UPEC ATCC 11775	S → I
	Y. pseudotuberculosis YPIII/pIB1	S → I
	Citrobacter freundii ATCC 8090	S → I
	Klebsiella pneumoniae ATCC 13883	S → R
4. MHB & DMEM have the same breakpoint & adding $NaHCO_3$ to MHB does not change this breakpoint		
All other drug & strains combinations not listed above		

* Previously published findings


a)
Daptomycin
Cephalothin
Ceftiofur
Ceftriaxone
Ampicillin
Oxacillin
Trimethoprim
Co-Trimoxazole
Spectinomycin
Streptomycin
Kanamycin
Gentamicin
Linezolid
Tetracycline
Azithromycin
Erythromycin
Chloramphenicol
Florfenicol
Clindamycin
Rifampin
Nalidixic Acid
Ciprofloxacin
Enrofloxacin
MRSA USA300 2 4 8 32 0 4 2 2 NA NA R 0 2 4 16 4 0 2 0 4 4 2 2
MRSA Blood 2 NA 0 4 4 4 0 2 NA NA R 0 2 4 16 8 0 2 2 4 8 2 0
MRSA Wound 0 8 0 2 4 0 0 2 NA NA R NA 2 4 16 2 0 2 0 4 2 2 2
MSSA Blood 0 0 4 2 0 4 2 2 0 NA 2 0 0 2 R R 0 2 0 2 4 2 4
MSSA Wound 0 4 4 2 4 8 2 0 0 NA 2 0 2 4 32 8 0 2 2 4 8 2 2
MSSA Urine 0 2 4 4 4 8 2 2 0 4 2 2 0 2 16 4 0 0 2 4 4 2 2
MSSA Sputum 0 0 2 2 2 4 2 4 0 2 4 0 0 4 32 16 0 2 2 2 8 2 2
CoN S. lugdunensis 0 2 4 4 32 2 2 NA 0 NA NA NA 0 2 NA 2 0 NA 2 2 8 0 2
CoN S. epidermidis 0 2 2 2 4 8 2 0 NA NA 4 NA 2 4 32 8 0 2 2 2 8 4 2
CoN S. warneri 2 2 2 0 0 2 0 NA 0 NA NA NA 0 2 64 32 0 2 2 8 8 0 NA
b)
Capsular serotype
Daptomycin
Ceftriaxone
Ampicillin
Oxacillin
Trimethoprim
Co-Trimoxazole
Spectinomycin
Linezolid
Tetracycline
Azithromycin
Erythromycin
Chloramphenicol
Clindamycin
Rifampin
Ciprofloxacin
D39 2 2 0 4 0 2 0 2 0 2 16 8 2 4 0 2
Daw 1 6 NA 4 2 2 2 2 0 2 2 32 8 0 2 0 0
Daw 19 6 2 NA 2 2 2 2 2 2 2 32 4 0 4 0 0
Daw 20 11 2 2 2 2 2 0 2 2 2 16 2 2 2 2 NA
Daw 2 23 2 2 4 2 4 2 0 2 0 16 4 0 2 2 2
Daw 25 35C 2 0 0 0 2 2 2 0 0 32 4 0 4 0 0
8+ ≥8-fold resistant
4 4-fold resistant
2 2-fold resistant
= no change
2 2-fold susceptible
4 4-fold susceptible
8+ ≥8-fold susceptible
R Resistant

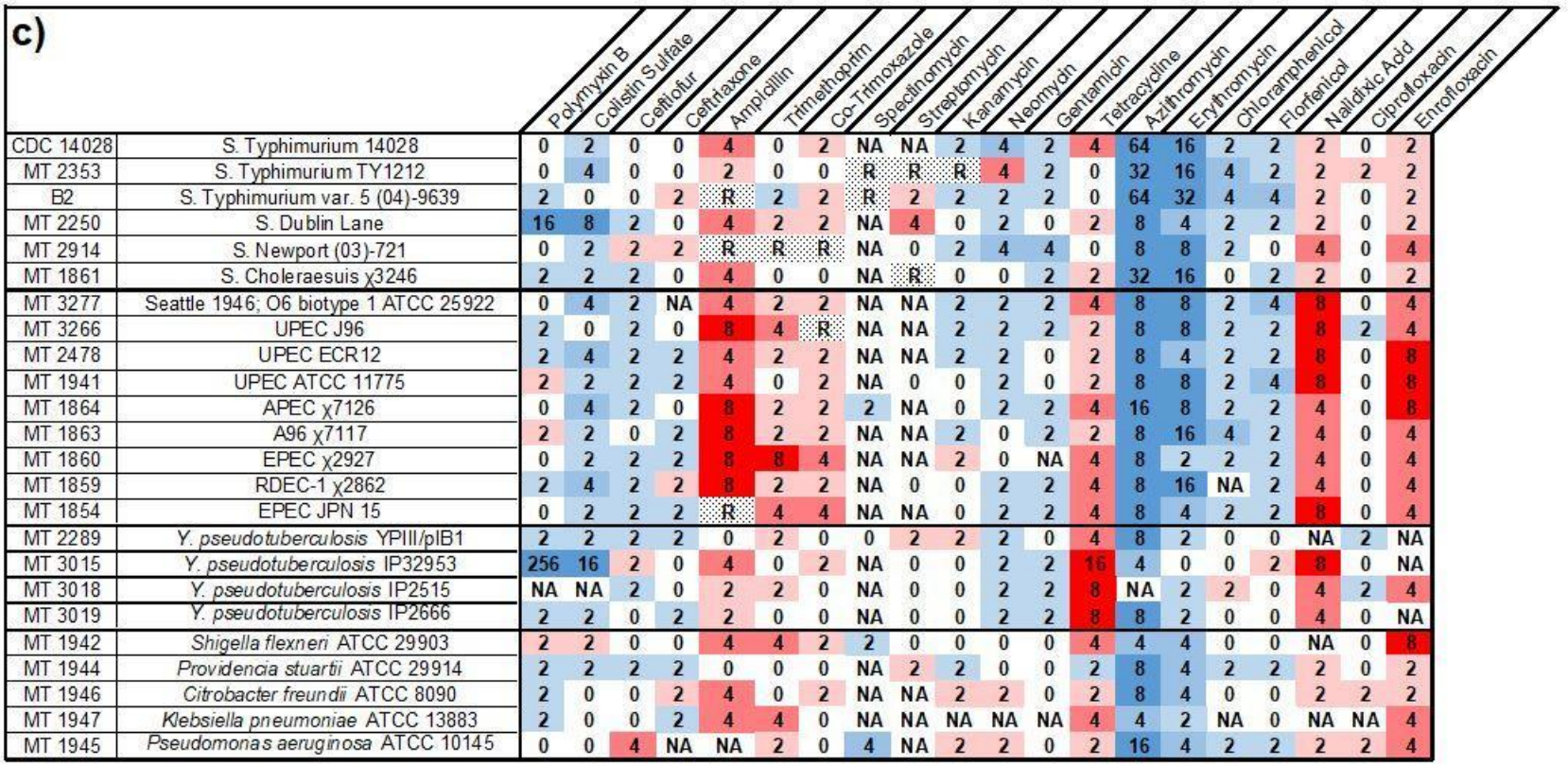


Figure 5. Fold change comparison of MIC values in MHB supplemented with 44mM $NaHCO_3$, relative to standard MHB. A panel of antibiotics was screened for changes in MIC values for (a) *Staphylococcus* spp., (b) *Streptococcus pneumoniae*, and (c) Gram-negative bacteria. Values depict the fold-change in MICs when AST was performed in MHB supplemented with 100mM Tris buffer and 44mM $NaHCO_3$, relative to standard MHB. Fold changes listed as "Not Applicable" (NA) were disregarded due to an effect of Tris buffer on antimicrobial susceptibility. MIC values were obtained from at least 6 independent determinations

Table 5a. Antimicrobial susceptibility test in media w/ $NaHCO_3$ (*Staphylococcus*)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

Strain #	Strain Name	Daptomycin (µg/mL)				Cephalothin (µg/mL)				Ceftiofur (µg/mL)				Ceftriaxone (µg/mL)			
Clinical Breakpoints[a]:		S ≤ 1; NS ≥ 2				S ≤ 8; I = 16; R ≥ 32[2]				S ≤ 2; I = 4; R ≥ 8[3]				S ≤ 8; I =16-32; R ≥ 64[2]			
		Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	1	1	0.5	0.5	32[b]	32[b]	8[b]	4	64[b]	128[b]	8[b]	8	512[b]	256[b]	16[b]	16
MT3302	MRSA Blood	0.5	0.5	0.25	0.5	8	NA	NA	2	16	32	16	4	128[b]	64[b]	32[b]	16
MT3315	MRSA Wound	0.5	1	0.5	0.25	8[b]	4[b]	1[b]	0.5	16	32	16	4	64[b]	64[b]	32[b]	8
MT3305	MSSA Blood	0.5	1	0.5	0.5	0.5	0.5	0.5	0.25	1	2	4	2	4	8	8	2
MT3307	MSSA Wound	0.5	0.5	0.5	0.5	0.125	0.25	0.5	0.0625	1	2	4	1	4	8	8	2
MT3309	MSSA Urine	0.5	1	0.5	1	0.25	0.5	0.5	0.125	1	2	4	1	4	8	16	2
MT3314	MSSA Sputum	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.125	1	1	2	1	4	4	8	2
MT3317	CoN S. lugdunensis	0.25	0.5	0.25	0.125	0.5	0.25	0.25	0.5	0.5	0.5	2	2	2	4	8	4
MT3320	CoN S. epidermidis	0.5	1	0.5	0.5	0.125	0.25	0.25	0.25	0.5	0.5	1	0.5	2	2	4	2
MT3321	CoN S. warneri	0.5	1	0.25	0.125	0.125	0.125	0.0625	0.03125	0.25	0.25	0.5	0.5	2	2	2	0.5

Strain #	Strain Name	Ampicillin (µg/mL)				Oxacillin (µg/mL)				Trimethoprim (µg/mL)				Co-Trimoxazole (µg/mL)			
Clinical Breakpoints:		S ≤ 0.25; R ≥ 0.5[2]				S ≤ 2; R ≥ 4				S ≤ 8; R ≥ 16				S ≤ 2/38; R ≥ 4/76			
		Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	128	256	128	256	64	128	256	16	2	1	1	2	0.0625/1.2	0.0625/1.2	0.125/2.4	0.125/2.4
MT3302	MRSA Blood	256	256	64	128	64[b]	64[b]	16[b]	4	2	4	2	4	0.125/2.4	0.125/2.4	0.25/4.75	0.125/2.4
MT3315	MRSA Wound	8[b]	8[b]	2[b]	0.5	32[b]	64[b]	32[b]	1	2	2	2	4	0.0625/1.2	0.0625/1.2	0.125/2.4	0.125/2.4
MT3305	MSSA Blood	2	2	2	2	0.25	0.5	1	0.25	1	2	2	2	0.0625/1.2	0.0625/1.2	0.125/2.4	0.125/2.4
MT3307	MSSA Wound	0.125	0.125	0.5	0.125	0.25	0.5	2	0.125	2[b]	1[b]	1[b]	>512	0.125/2.4[b]	0.0625/1.2[b]	0.125/2.4[b]	>64/1216
MT3309	MSSA Urine	0.125	0.25	0.5	0.125	0.25	0.5	2	0.125	1	2	2	1	0.0625/1.2	0.0625/1.2	0.125/2.4	0.125/2.4
MT3314	MSSA Sputum	0.125	0.125	0.25	0.125	0.25	0.25	1	0.125	2	4	4	1	0.0625/1.2	0.0625/1.2	0.25/4.75	0.125/2.4
MT3317	CoN S. lugdunensis	128	64	4		0.5	0.5	1	0.5	16	32	32	16	0.25/4.75	NA	NA	0.5/9.5
						Clinical Breakpoints: S ≤ 0.25; R ≥ 0.5											
MT3320	CoN S. epidermidis	8	8	2	16	0.125	0.25	1	0.25	1	1	2	1	0.25/4.75	0.125/2.4	0.25/4.75	0.125/2.4
MT3321	CoN S. warneri	0.03125	0.0625	0.03125	0.0156	0.125	0.25	0.25	0.125	2	4	2	2	0.0625/1.2	NA	NA	0.125/2.4

Strain #	Strain Name	Spectinomycin (µg/mL)				Streptomycin (µg/mL)				Kanamycin (µg/mL)				Gentamicin (µg/mL)			
Clinical Breakpoints:						S ≤ 8; I = 16; R ≥ 32[4]				S ≤ 16; I = 32; R ≥ 64				S ≤ 4; I = 8; R ≥ 16			
		Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	128	NA	NA	64	16	NA	NA	4	>512	>512	>512	>512	1	2	1	2
MT3302	MRSA Blood	128	NA	NA	32	8	NA	NA	2	>512	>512	>512	>512	1	2	1	4
MT3315	MRSA Wound	128	NA	NA	64	8	NA	NA	1	>512	>512	>512	>512	128	NA	NA	>512
MT3305	MSSA Blood	128	256	128	64	16	NA	NA	2	8	8	16	16	1	2	1	2
MT3307	MSSA Wound	256	256	256	64	16	NA	NA	4	8	8	16	16	1	2	1	4
MT3309	MSSA Urine	128	256	128	64	32	32	8	4	4	8	8	8	0.5	1	1	2
MT3314	MSSA Sputum	256	512	256	64	32	32	16	2	4	8	16	8	1	2	1	2
MT3317	CoN S. lugdunensis	64	128	64	32	4	NA	NA	1	1	NA	NA	4	0.125	NA	NA	0.5
MT3320	CoN S. epidermidis	128	NA	NA	64	4	NA	NA	0.5	2	4	8	8	0.125	NA	NA	0.5
MT3321	CoN S. warneri	64	128	64	32	4	NA	NA	1	1	NA	NA	4	0.125	NA	NA	0.5

		Linezolid (μg/mL)				Tetracycline (μg/mL)				Azithromycin (μg/mL)				Erythromycin (μg/mL)			
Clinical Breakpoints:		S ≤ 4; R ≥ 8				S ≤ 4; I = 8; R ≥ 16				S ≤ 2; I = 4; R ≥ 8				S ≤ 0.5; I = 1 - 4; R ≥ 8			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	2	4	4	4	0.5[b]	0.5[b]	2[b]	4	128[b]	128[b]	8[b]	8	64[b]	64[b]	16[b]	8
MT3302	MRSA Blood	2	4	4	2	0.5	0.5	2	2	128[b]	256[b]	8[b]	8	128[b]	128[b]	16[b]	8
MT3315	MRSA Wound	2	4	4	2	0.5	0.5	2	1	1[b]	2[b]	0.0625[b]	0.125	0.25	0.5	0.125	0.0625
MT3305	MSSA Blood	2	4	2	2	0.5	0.5	1	0.5	>512	>512	>512	>512	>512	>512	>512	>512
MT3307	MSSA Wound	2	4	4	2	0.5[b]	0.5[b]	2[b]	4	2[b]	2[b]	0.0625[b]	0.125	1[b]	0.5[b]	0.125[b]	0.125
MT3309	MSSA Urine	2	4	2	2	4	4	8	8	1[b]	2[b]	0.0625[b]	0.125	0.5	0.5	0.125	0.125
MT3314	MSSA Sputum	2	4	2	2	0.5	0.5	2	2	2[b]	2[b]	0.0625[b]	0.125	1[b]	0.5[b]	0.0625[b]	0.125
MT3317	CoN S. lugdunensis	1	2	1	1	0.25	0.25	0.5	1	0.25	NA	NA	0.0625	0.125	0.25	0.0625	0.03125
MT3320	CoN S. epidermidis	2	4	4	2	0.5[b]	0.25[b]	2[b]	4	256[b]	256[b]	8[b]	32	128	128	16	32
MT3321	CoN S. warneri	2	4	2	2	0.5[b]	0.25[b]	1[b]	4	256[b]	128[b]	4[b]	32	256[b]	128[b]	8[b]	32

		Chloramphenicol (μg/mL)				Florfenicol (μg/mL)				Clindamycin (μg/mL)				Rifampin (μg/mL)			
Clinical Breakpoints:		S ≤ 8; I = 16; R ≥ 32								S ≤ 0.5; I = 1 - 2; R ≥ 4				S ≤ 1; I = 2; R ≥ 4			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	8	8	8	8	4	8	8	4	0.125	0.25	0.125	0.0625	0.0078	0.0078	0.03125	0.0156
MT3302	MRSA Blood	8	8	8	4	4	8	8	4	0.25	0.25	0.125	0.125	0.0078	0.0078	0.03125	0.0156
MT3315	MRSA Wound	8	8	8	8	4	8	8	4	0.125	0.25	0.125	0.125	0.0078	0.0078	0.03125	0.03125
MT3305	MSSA Blood	8	8	8	8	4	8	8	4	0.25	0.25	0.25	0.125	0.0156	0.0156	0.03125	0.0156
MT3307	MSSA Wound	8	8	8	16	4	8	8	4	0.25	0.25	0.125	0.0625	0.0078	0.0078	0.03125	0.0156
MT3309	MSSA Urine	8	8	8	4	8	8	8	4	0.25	0.25	0.125	0.125	0.0078	0.0156	0.03125	0.0156
MT3314	MSSA Sputum	8	8	8	4	4	8	8	4	0.25	0.25	0.125	0.125	0.0156	0.0156	0.03125	0.0156
MT3317	CoN S. lugdunensis	4	8	4	8	2	NA	NA	4	0.125	0.25	0.0625	0.03125	0.0078	0.0078	0.0156	0.0156
MT3320	CoN S. epidermidis	8	16	8	8	4	8	8	8	0.25	0.5	0.125	0.125	0.0078	0.0078	0.0156	0.03125
MT3321	CoN S. warneri	8	16	8	8	4	8	8	4	0.125	0.25	0.0625	0.0625	0.002	0.004	0.0156	0.0078

		Nalidixic Acid (μg/mL)				Ciprofloxacin (μg/mL)				Enrofloxacin (μg/mL)			
Clinical Breakpoints:						S ≤ 1; I = 2; R ≥ 4							
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LB pH 7.4 [b]
MT3322	MRSA USA300	64	64	256	256	0.5	0.5	1	0.5	0.125	0.125	0.25	0.25
MT3302	MRSA Blood	64	64	512	256	0.5	1	1	0.25	0.25	0.25	0.25	0.25
MT3315	MRSA Wound	256	256	512	>512	16	32	32	16	4	4	8	4
MT3305	MSSA Blood	64	64	256	128	0.25	0.25	0.5	0.25	0.0625	0.125	0.25	0.25
MT3307	MSSA Wound	32[b]	32[b]	256[b]	256	0.25	0.25	0.5	0.5	0.125	0.125	0.25	0.25
MT3309	MSSA Urine	256	256	1024	>512	16	16	32	16	4	4	8	8
MT3314	MSSA Sputum	32	32	256	128	0.25	0.25	0.5	0.25	0.125	0.125	0.25	0.25
MT3317	CoN S. lugdunensis	64[b]	128[b]	512[b]	512	0.25	0.25	0.25	0.25	0.125	0.25	0.25	0.25
MT3320	CoN S. epidermidis	32[b]	64[b]	256[b]	256	0.125	0.25	0.5	0.25	0.125	0.25	0.25	0.25
MT3321	CoN S. warneri	256	512	2048	512	0.25	0.5	0.25	0.5	0.25	NA	NA	0.5

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant
Values marked with "NA" were disregarded due to an effect of Tris buffer on antimicrobial susceptibility.

[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated

[b] Previously published values, obtained in a separate experiment

Table 5b. Antimicrobial susceptibility test in media w/ $NaHCO_3$ (*Streptococcus pneumoniae*)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

		Daptomycin (µg/mL)				Ceftriaxone (µg/mL)				Ampicillin (µg/mL)				Oxacillin (µg/mL)			
Clinical Breakpoints[a]:		S ≤ 2; NS ≥4[5]				S ≤ 1; I = 2; R ≥ 4				S ≤ 0.5; I = 1-2; R ≥ 4[6]							
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]
D39	2	0.25	0.5	0.5	0.0625	0.03125	0.03125	0.03125	0.0156	0.015625	0.015625	0.0625	0.03125	0.125	0.125	0.125	0.125
Daw 1	6	0.25	NA	NA	0.0625	0.5	1	2	0.5	2[b]	2[b]	4[b]	0.0625	8	8	16	8
Daw 19	6	0.25	0.5	0.5	0.0625	1	NA	NA	0.5	8	16	16	0.0625	8	16	16	8
Daw 20	11	0.25	0.5	0.5	0.0625	0.03125	0.03125	0.0625	0.03125	0.03125	0.03125	0.0625	0.0625	0.0625	0.125	0.125	0.25
Daw 2	23	0.25	0.5	0.5	0.0625	2[b]	2[b]	4[b]	4	0.5[b]	1[b]	2[b]	4	2	4	4	8
Daw 25	35C	0.25	0.5	0.5	0.0625	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.0625	0.0625	0.0625	0.0625	0.125

		Trimethoprim (µg/mL)				Co-Trimoxazole (µg/mL)				Spectinomycin (µg/mL)				Linezolid (µg/mL)			
Clinical Breakpoints:		S ≤ 2; R ≥ 4[7]				S ≤ 0.5/9.5; I = 1/19 - 2/38; R ≥ 4/76								S ≤ 2; NS ≥ 4			
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]
D39	2	1	1	0.5	0.5	0.125/2.4	0.125/2.4	0.125/2.4	0.125/2.4	64	64	32	8	1	1	1	1
Daw 1	6	256	256	128	2	8/152	8/152	4/76	1/19	32	64	32	8	1	2	2	0.5
Daw 19	6	128	128	64	16	8/152	4/76	4/76	4/76	32	64	16	16	2	2	1	2
Daw 20	11	2	2	1	2	0.25/4.75	0.25/4.75	0.25/4.75	0.5/9.5	64	64	32	16	1	2	2	1
Daw 2	23	64	64	16	8	4/76	4/76	2/38	2/38	32	64	32	16	1	2	2	2
Daw 25	35C	4	4	2	2	0.25/4.75	0.25/4.75	0.125/2.4	0.25/4.8	32	64	16	16	1	1	1	1

		Tetracycline (μg/mL)				Azithromycin (μg/mL)				Erythromycin (μg/mL)				Chloramphenicol (μg/mL)			
Clinical Breakpoints:		S ≤ 1; I = 2; R ≥ 4				S ≤ 0.5; I = 1; R ≥ 2				S ≤ 0.25; I = 0.5; R ≥ 1				S ≤ 4; R ≥ 8			
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]
D39	2	0.125	0.25	0.25	0.5	0.25[b]	0.25[b]	0.0156[b]	0.0039	0.125[b]	0.125[b]	0.0156[b]	0.0039	4	4	2	1
Daw 1	6	0.25	0.5	0.5	1	64	64	2	2	16	16	2	2	4	4	4	2
Daw 19	6	0.25	0.5	0.5	1	64	64	2	2	16	16	4	2	4	4	4	2
Daw 20	11	0.125	0.25	0.25	0.5	0.5	0.5	0.03125	0.0156	0.0625	0.0625	0.03125	0.0156	2	4	4	2
Daw 2	23	0.25	0.25	0.25	0.5	0.5	0.5	0.03125	0.0156	0.125	0.125	0.03125	0.0156	4	4	4	4
Daw 25	35C	0.125	0.125	0.125	0.5	0.5	0.5	0.0156	0.0156	0.0625	0.0625	0.0156	0.0078	2	2	2	2

		Clindamycin (μg/mL)				Rifampin (μg/mL)				Ciprofloxacin (μg/mL)			
Clinical Breakpoints:		S ≤ 0.25; I = 0.5; R ≥ 1				S ≤ 1; I = 2; R ≥ 4				S ≤ 0.125; I = 0.25-2; R ≥ 4[6]			
Strain	Capsular Serotype	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]	Ca-MHB + 5% LHB pH 7.2	Ca-MHB + 5% LHB + 100mM Tris pH 7.2	Ca-MHB + 5% LHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM + 5% LHB pH 7.4 [b]
D39	2	0.0625	0.0625	0.0156	0.0078	0.03125	0.03125	0.03125	0.03125	0.5	1	1	1
Daw 1	6	0.125	0.125	0.0625	0.03125	0.0156	0.0156	0.0156	0.0156	1	2	1	2
Daw 19	6	0.125	0.125	0.03125	0.03125	0.0156	0.0156	0.0156	0.03125	1	1	1	1
Daw 20	11	0.125	0.125	0.0625	0.0625	0.03125	0.03125	0.0625	0.0625	2	NA	NA	2
Daw 2	23	0.125	0.25	0.0625	0.0625	0.0156	0.0156	0.03125	0.0625	1	2	2	2
Daw 25	35C	0.125	0.125	0.03125	0.03125	0.0156	0.0156	0.0156	0.0625	1	1	1	1

All MICs were determined by broth microdilution in accordance with CLSI guidelines. MIC plates were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant
Values marked with "NA" were disregarded due to an effect of Tris buffer on antimicrobial susceptibility.

[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated

[b] Previously published values, obtained in a separate experiment

Table 5c. Antimicrobial susceptibility test in media w/ $NaHCO_3$ (Gram-negative bacteria)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

		Polymyxin B (µg/mL)				Colistin Sulfate (µg/mL)				Ceftiofur (µg/mL)				Ceftriaxone (µg/mL)			
	Clinical Breakpoints[a]:	S ≤ 2, R ≥ 4[b]				S ≤ 2, R ≥ 4[b]				S ≤ 2, I = 4, R ≥ 8[b]				S ≤ 1, I = 2, R ≥ 4			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]
CDC 14028	S. Typhimurium 14028	0.25	0.5	0.25	2	0.5	0.5	0.25	4	0.5	0.5	0.5	0.5	0.125	0.125	0.125	0.0625
MT 2353	S. Typhimurium TY1212	0.25	0.5	0.25	2	0.5	1	0.125	2	1	1	1	0.5	0.25	0.125	0.25	0.125
B2	S. Typhimurium var. 5 (04)-9639	0.5	0.25	0.25	1	0.25	0.5	0.25	2	1	1	1	0.5	0.125	0.125	0.25	0.0625
MT 2250	S. Dublin Lane	4	4	0.25	2	4	8	0.5	8	1	0.5	0.5	0.125	0.0625	0.0625	0.0625	0.0156
MT 2914	S. Newport (03)-721	0.25	0.5	0.25	2	0.5	1	0.25	2	128	128	256	128	128	64	256	64
MT 1861	S. Choleraesuis χ3246	0.25	0.25	0.125	1	0.25	0.25	0.125	2	2	2	1	0.5	0.125	0.125	0.125	0.03125
	Clinical Breakpoints:	S ≤ 2, R ≥ 4[b]				S ≤ 2, R ≥ 4[b]				S ≤ 2, I = 4, R ≥ 8[b]				S ≤ 1, I = 2, R ≥ 4			
MT 3277	Seattle 1946; O6 biotype 1 ATCC 25922	0.5	0.5	0.5	1	1	1	0.25	1	0.5	0.25	0.25	0.25	0.125	NA	NA	0.03125
MT 3266	UPEC J96	0.5	0.5	0.25	1	0.5	0.5	0.5	1	0.5	0.25	0.25	0.25	0.0625	0.0625	0.0625	0.03125
MT 2478	UPEC ECR12	0.5	0.5	0.25	1	1	0.5	0.25	1	0.5	0.25	0.25	0.5	0.0625	0.0625	0.03125	0.0625
MT 1941	UPEC ATCC 11775	0.125	0.25	0.25	1	0.5	0.25	0.25	1	0.5	0.25	0.25	0.25	0.0625	0.03125	0.03125	0.03125
MT 1864	APEC χ7126	0.125	0.25	0.125	2	0.5	0.25	0.125	1	0.25	0.125	0.125	0.125	0.03125	0.03125	0.03125	0.0156
MT 1863	A96 χ7117	0.125	0.25	0.25	1	0.5	0.25	0.25	1	0.25	0.25	0.25	0.125	0.0625	0.0625	0.03125	0.03125
MT 1860	EPEC χ2927	0.25	0.25	0.25	1	0.25	0.25	0.125	1	0.5	0.5	0.25	0.25	0.0625	0.0625	0.03125	0.0156
MT 1859	RDEC-1 χ2862	0.5	0.25	0.25	0.5	0.5	0.25	0.125	1	0.5	0.25	0.25	0.25	0.0625	0.0625	0.125	0.03125
MT 1854	EPEC JPN 15	0.25	0.25	0.25	1	0.5	0.25	0.25	1	1	0.5	0.5	0.5	0.125	0.0625	0.0625	0.03125
	Clinical Breakpoints:	S ≤ 2, R ≥ 4[b]				S ≤ 2, R ≥ 4[b]				S ≤ 2, I = 4, R ≥ 8[b]				S ≤ 1, I = 2, R ≥ 4			
MT 2289	*Y. pseudotuberculosis* YPIII/pIB1	0.25	0.125	0.125	1	0.25	0.125	0.125	1	0.125	0.125	0.0625	0.125	0.03125	0.0156	0.0156	0.0156
MT 3015	*Y. pseudotuberculosis* IP32953	128	256	0.5	128	256	512	16	256	0.125	0.25	0.25	0.125	0.03125	0.0625	0.03125	0.0156
MT 3018	*Y. pseudotuberculosis* IP2515	64	NA	NA	128	128	NA	NA	256	0.125	0.125	0.0625	0.125	0.0156	0.0156	0.0156	0.0156
MT 3019	*Y. pseudotuberculosis* IP2666	0.25	0.125	0.125	1	0.25	0.125	0.125	1	0.125	0.25	0.125	0.0625	0.0156	0.0156	0.0078	0.0078
	Clinical Breakpoints:	S ≤ 2, R ≥ 4[b]				S ≤ 2, R ≥ 4[b]				S ≤ 2, I = 4, R ≥ 8[b]				S ≤ 1, I = 2, R ≥ 4			
MT 1942	*Shigella flexneri* ATCC 29903	0.25	0.5	0.5	1	0.25	0.5	0.5	1	0.25	0.25	0.25	0.125	0.03125	0.03125	0.03125	0.0156
MT 1944	*Providencia stuartii* ATCC 29914	32	64	16	256	64	128	32	512	0.25	0.25	0.125	0.5	0.0156	0.0156	0.0078	0.0625
MT 1946	*Citrobacter freundii* ATCC 8090	0.5	0.25	0.25	2	0.5	0.5	0.5	2	0.5	0.5	0.5	0.5	0.125	0.0625	0.25	0.0625
MT 1947	*Klebsiella pneumoniae* ATCC 13883	8	16	4	16	16	32	16	16	1	1	1	1	0.125	0.0625	0.0625	0.125
	Clinical Breakpoints:	S ≤ 2, I = 4, R ≥ 8				S ≤ 2, I = 4, R ≥ 8				S ≤ 2, I = 4, R ≥ 8[b]							
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1	1	1	8	1	1	1	16	32	64	128	32	8	NA	NA	8

		Ampicillin (µg/mL)				Trimethoprim (µg/mL)				Co-Trimoxazole (µg/mL)				Spectinomycin (µg/mL)			
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32				S ≤ 8, R ≥ 16				S ≤ 2/38, R ≥ 4/76				S ≤ 32; I = 64-128; R ≥ 256[10]			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]
CDC 14028	S. Typhimurium 14028	2	4	8	4	0.25	0.125	0.25	1	0.0625/1.2	0.03125/0.6	0.125/2.4	0.25/4.75	64	NA	NA	32
MT 2353	S. Typhimurium TY1212	4	8	8	4	0.5	0.25	0.5	2	0.25/4.75	0.125/2.4	0.25/4.75	1/19	>1024	>1024	>1024	>1024
B2	S. Typhimurium var. 5 (04)-9639	>2048	>2048	>2048	>2048	1	0.5	0.5	0.5	0.25/4.75	0.25/4.75	0.5/9.5	0.25/4.75	>1024	>1024	>1024	>1024
MT 2250	S. Dublin Lane	1	2	4	1	0.5	0.5	1	0.5	0.125/2.4	0.25/4.75	0.25/4.75	0.25/4.75	64	NA	NA	32
MT 2914	S. Newport (03)-721	2048	2048	>2048	>2048	>256	>256	>256	>256	>64/1216	>64/1216	>64/1216	>64/1216	128	NA	NA	64
MT 1861	S. Choleraesuis χ3246	2	4	8	2	0.25	0.125	0.25	0.5	0.25/4.75	0.125/2.4	0.25/4.75	0.5/9.5	64	NA	NA	32
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32				S ≤ 8, R ≥ 16				S ≤ 2/38, R ≥ 4/76				S ≤ 32; I = 64-128; R ≥ 256[10]			
MT 3277	Seattle 1946; O6 biotype 1 ATCC 25922	4	8	16	8	1	1	2	0.5	0.0625/1.2	0.125/2.4	0.125/2.4	0.03125/0.6	32	NA	NA	16
MT 3266	UPEC J96	4	8	32	4	1	2	4	0.5	0.0625/1.2	NA	NA	0.03125/0.6	32	NA	NA	16
MT 2478	UPEC ECR12	4	8	16	8	0.5	0.5	1	0.5	0.0625/1.2	0.125/2.4	0.125/2.4	0.0625/1.2	16	NA	NA	8
MT 1941	UPEC ATCC 11775	4	4	16	4	1	1	1	0.5	0.125/2.4	0.125/2.4	0.25/4.75	0.0625/1.2	16	NA	NA	16
MT 1864	APEC χ7126	2	4	16	2	0.25	0.25	0.5	0.25	0.0625/1.2	0.0625/1.2	0.125/2.4	0.0625/1.2	16	32	8	16
MT 1863	A96 χ7117	4	4	32	4	0.25	0.25	0.5	0.25	0.0625/1.2	0.0625/1.2	0.125/2.4	0.03125/0.6	32	NA	NA	16
MT 1860	EPEC χ2927	4	8	32	4	0.25	0.5	2	0.125	0.0625/1.2	0.125/2.4	0.25/4.75	0.03125/0.6	32	NA	NA	8
MT 1859	RDEC-1 χ2862	4	8	32	8	0.25	0.5	0.5	1	0.0625/1.2	0.0625/1.2	0.125/2.4	0.03125/0.6	16	NA	NA	4
MT 1854	EPEC JPN 15	>2048	>2048	>2048	>2048	0.5	1	2	0.5	0.0625/1.2	0.125/2.4	0.25/4.75	0.03125/0.6	16	NA	NA	16
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32				S ≤ 8, R ≥ 16				S ≤ 2/38, R ≥ 4/76				S ≤ 32; I = 64-128; R ≥ 256[10]			
MT 2289	*Y. pseudotuberculosis* YPIII/pIB1	0.25	0.25	0.25	0.25	4	4	8	0.0625	0.25/4.75	0.125/2.4	0.25/4.75	0.0156/0.3	16	32	16	8
MT 3015	*Y. pseudotuberculosis* IP32953	0.25	0.5	1	0.25	2	1	2	0.0625	0.125/2.4	0.125/2.4	0.25/4.75	0.0156/0.3	16	NA	NA	16
MT 3018	*Y. pseudotuberculosis* IP2515	0.25	0.25	0.5	0.25	1	1	2	0.0625	0.125/2.4	0.125/2.4	0.125/2.4	0.0156/0.3	16	NA	NA	8
MT 3019	*Y. pseudotuberculosis* IP2666	0.25	0.25	0.5	0.125	2	2	2	0.0625	0.125/2.4	0.125/2.4	0.125/2.4	0.0156/0.3	16	NA	NA	16
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32				S ≤ 8, R ≥ 16				S ≤ 2/38, R ≥ 4/76				S ≤ 32; I = 64-128; R ≥ 256[10]			
MT 1942	*Shigella flexneri* ATCC 29903	2	2	8	2	0.25	0.5	1	0.25	0.25/4.75	0.5/9.5	0.5/9.5	0.125/2.4	64	128	32	16
MT 1944	*Providencia stuartii* ATCC 29914	32	32	32	64	2	2	2	2	0.25/4.75	0.25/4.75	0.25/4.75	0.0625/1.2	>1024	NA	NA	>1024
MT 1946	*Citrobacter freundii* ATCC 8090	16	16	64	128	1	0.5	1	1	0.0625/1.2	0.125/2.4	0.125/2.4	0.0625/1.2	32	NA	NA	16
MT 1947	*Klebsiella pneumoniae* ATCC 13883	256	256	1024	256	2	4	8	1	0.25/4.75	0.25/4.75	0.25/4.75	0.03125/0.6	16	NA	NA	8
	Clinical Breakpoints:																
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	128	NA	NA	256	512	1024	1024	>512	32/608	32/608	32/608	32/608	2048	2048	512	512

		Streptomycin (μg/mL)				Kanamycin (μg/mL)				Neomycin (μg/mL)				Gentamicin (μg/mL)			
Clinical Breakpoints:		S ≤ 8; I = 16, R ≥ 32[4]				S ≤ 16, I = 32, R ≥ 64				S ≤ 8, I = 16, R ≥ 32[11]				S ≤ 4, I = 8, R ≥ 16			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]
CDC 14028	S. Typhimurium 14028	16	NA	NA	8	4	4	2	8	2	2	0.5	8	1	1	0.5	2
MT 2353	S. Typhimurium TY1212	>1024	>1024	1024	512	2048	2048	>2048	>2048	256	512	1024	1024	1	1	0.5	2
B2	S. Typhimurium var. 5 (04)-9639	256	512	512	64	4	4	2	8	2	2	1	4	1	1	0.5	1
MT 2250	S. Dublin Lane	32	64	128	8	2	2	2	8	1	1	0.5	2	0.5	1	0.5	1
MT 2914	S. Newport (03)-721	1024	2048	1024	1024	4	4	2	8	2	2	0.5	8	1	1	0.25	1
MT 1861	S. Choleraesuis χ3246	2048	>2048	>2048	512	4	4	4	4	1	2	1	2	1	1	0.5	1
Clinical Breakpoints:		S ≤ 8; I = 16, R ≥ 32[4]				S ≤ 16, I = 32, R ≥ 64				S ≤ 8, I = 16, R ≥ 32[11]				S ≤ 4, I = 8, R ≥ 16			
MT 3277	Seattle 1946; O6 biotype 1 ATCC 25922	8	NA	NA	2	4	4	2	8	2	1	1	4	1	1	0.5	1
MT 3266	UPEC J96	4	NA	NA	1	4	4	2	4	2	2	1	2	1	1	0.5	1
MT 2478	UPEC ECR12	8	NA	NA	2	4	4	2	8	2	2	1	4	0.5	1	0.5	1
MT 1941	UPEC ATCC 11775	4	8	4	2	2	2	2	8	1	1	0.5	4	0.5	0.5	0.5	1
MT 1864	APEC χ7126	8	NA	NA	2	4	4	4	8	2	2	1	2	1	1	0.5	1
MT 1863	A96 χ7117	8	NA	NA	2	4	4	2	8	1	2	1	4	1	1	0.5	1
MT 1860	EPEC χ2927	4	NA	NA	1	1	2	2	2	0.5	1	0.5	1	0.25	NA	NA	0.5
MT 1859	RDEC-1 χ2862	4	8	4	1	2	2	2	2	1	1	0.5	1	0.5	0.5	0.25	0.5
MT 1854	EPEC JPN 15	8	NA	NA	4	2	2	2	8	1	0.5	0.5	4	0.5	0.5	0.25	1
Clinical Breakpoints:		S ≤ 8; I = 16, R ≥ 32[4]				S ≤ 16, I = 32, R ≥ 64				S ≤ 8, I = 16, R ≥ 32[11]				S ≤ 4, I = 8, R ≥ 16			
MT 2289	*Y. pseudotuberculosis* YPIII/pIB1	1	2	2	0.5	0.25	0.25	0.5	2	0.5	0.5	0.25	1	0.125	0.125	0.125	0.5
MT 3015	*Y. pseudotuberculosis* IP32953	4	8	4	1	1	2	1	4	1	1	0.5	2	0.5	0.5	0.25	2
MT 3018	*Y. pseudotuberculosis* IP2515	2	4	2	0.5	0.5	1	0.5	2	0.5	0.5	0.25	1	0.25	0.25	0.125	1
MT 3019	*Y. pseudotuberculosis* IP2666	2	4	2	1	0.5	0.5	0.5	4	0.5	0.5	0.25	2	0.25	0.125	0.125	1
Clinical Breakpoints:		S ≤ 8; I = 16, R ≥ 32[4]				S ≤ 16, I = 32, R ≥ 64				S ≤ 8, I = 16, R ≥ 32[11]				S ≤ 4, I = 8, R ≥ 16			
MT 1942	*Shigella flexneri* ATCC 29903	8	16	8	2	4	4	4	8	4	4	4	4	1	2	1	2
MT 1944	*Providencia stuartii* ATCC 29914	64	128	128	128	0.5	0.5	0.25	4	0.5	0.5	0.5	8	1	1	1	2
MT 1946	*Citrobacter freundii* ATCC 8090	4	NA	NA	1	2	4	4	8	1	2	2	4	0.5	1	0.5	1
MT 1947	*Klebsiella pneumoniae* ATCC 13883	2	NA	NA	1	1	NA	NA	4	0.5	NA	NA	2	0.25	NA	NA	0.5
Clinical Breakpoints:														S ≤ 4, I = 8, R ≥ 16			
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	64	NA	NA	32	64	64	128	512	16	16	32	256	4	4	4	32

		Tetracycline (μg/mL)				Azithromycin (μg/mL)				Erythromycin (μg/mL)				Chloramphenicol (μg/mL)			
Clinical Breakpoints:		S ≤ 4, I = 8, R ≥ 16				S ≤ 16, R ≥ 32[6]								S ≤ 8, I = 16, R ≥ 32			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]
CDC 14028	S. Typhimurium 14028	1[b]	1[b]	4[b]	8	8[b]	8[b]	0.125[b]	2	128[b]	128[b]	8[b]	32	8	8	4	4
MT 2353	S. Typhimurium TY1212	256	128	256	128	8	16	0.25	2	128	128	8	64	512	512	128	256
B2	S. Typhimurium var. 5 (04)-9639	64	64	64	64	8	8	0.125	2	128	128	4	64	256	256	64	256
MT 2250	S. Dublin Lane	2	2	4	4	4	4	0.5	1	64	64	16	32	8	8	4	2
MT 2914	S. Newport (03)-721	256	256	256	128	4	8	0.5	2	128	128	16	32	256	256	128	256
MT 1861	S. Choleraesuis χ3246	1[b]	1[b]	2[b]	8	8[b]	8[b]	0.25[b]	2	128[b]	64[b]	8[b]	32	4	4	4	2
Clinical Breakpoints:		S ≤ 4, I = 8, R ≥ 16				S ≤ 16, R ≥ 32[6]								S ≤ 8, I = 16, R ≥ 32			
MT 3277	Seattle 1946; O6 biotype 1 ATCC 25922	0.5	1	2	8	4	4	0.5	2	64	64	8	64	4	4	2	4
MT 3266	UPEC J96	1	1	2	8	4	8	0.5	1	64	64	8	64	8	8	4	8
MT 2478	UPEC ECR12	2	2	4	8	4	4	0.5	2	64	64	16	64	8	8	4	8
MT 1941	UPEC ATCC 11775	1	1	2	8	4	4	0.5	2	64	32	8	64	4	4	2	4
MT 1864	APEC χ7126	0.5	1	2	4	4	4	0.25	2	32	32	4	64	4	4	2	4
MT 1863	A96 χ7117	1	1	2	4	4	4	0.5	2	64	64	4	64	8	4	2	4
MT 1860	EPEC χ2927	0.5	1	2	4	4	8	0.5	1	32	64	16	32	8	8	4	4
MT 1859	RDEC-1 χ2862	2	4	8	8	8	8	1	4	256	256	16	128	8	NA	NA	8
MT 1854	EPEC JPN 15	0.5	1	2	4	4	4	0.5	2	64	64	16	32	8	8	4	8
Clinical Breakpoints:		S ≤ 4, I = 8, R ≥ 16				S ≤ 16, R ≥ 32[6]								S ≤ 8, I = 16, R ≥ 32			
MT 2289	*Y. pseudotuberculosis* YPIII/pIB1	1	1	4	8	8	8	1	2	64	64	32	32	8	8	8	8
MT 3015	*Y. pseudotuberculosis* IP32953	1	2	16	8	8	16	2	2	64	128	64	32	8	8	8	8
MT 3018	*Y. pseudotuberculosis* IP2515	1	2	8	8	4	NA	NA	2	64	128	32	64	4	8	8	4
MT 3019	*Y. pseudotuberculosis* IP2666	1	2	8	8	8	8	1	2	64	64	32	32	8	8	8	8
Clinical Breakpoints:		S ≤ 4, I = 8, R ≥ 16				S ≤ 16, R ≥ 32[6]								S ≤ 8, I = 16, R ≥ 32			
MT 1942	*Shigella flexneri* ATCC 29903	1	1	4	4	2	2	0.5	1	32	32	8	16	2	2	2	1
MT 1944	*Providencia stuartii* ATCC 29914	128	64	64	64	32	64	4	16	256	256	64	128	32	32	16	32
MT 1946	*Citrobacter freundii* ATCC 8090	1	1	2	8	8	16	1	8	256	256	64	128	4	8	4	8
MT 1947	*Klebsiella pneumoniae* ATCC 13883	1	2	4	16	8	8	2	4	64	128	32	64	8	NA	NA	8
Clinical Breakpoints:																	
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	64	32	128	>512	256	256	16	32	512	512	128	128	256	256	128	128

		Florfenicol (µg/mL)				Nalidixic Acid (µg/mL)				Ciprofloxacin (µg/mL)				Enrofloxacin (µg/mL)			
Clinical Breakpoints:		S ≤ 4, I = 8, R ≥ 16[3]				S ≤ 16, R ≥ 32				S ≤ 0.0625, I = 0.125-0.5, R ≥ 1				S ≤ 0.5; I = 1; R ≥ 2[12]			
Strain #	Strain Name	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]	Ca-MHB pH 7.2	Ca-MHB + 100mM Tris pH 7.2	Ca-MHB + 100mM Tris + 44mM $NaHCO_3$ pH 7.4	DMEM pH 7.4 [b]
CDC 14028	S. Typhimurium 14028	8	8	4	4	4	4	8	8	0.0156	0.03125	0.0156	0.0078	0.03125	0.0625	0.0625	0.0625
MT 2353	S. Typhimurium TY1212	512	512	256	256	4	8	8	16	0.0156	0.03125	0.03125	0.0078	0.0625	0.0625	0.125	0.125
B2	S. Typhimurium var. 5 (04)-9639	64	64	16	64	4	4	8	8	0.0156	0.03125	0.0156	0.0078	0.03125	0.03125	0.0625	0.0625
MT 2250	S. Dublin Lane	4	NA	NA	2	4	4	8	8	0.0156	0.03125	0.0156	0.0039	0.0625	0.0625	0.125	0.03125
MT 2914	S. Newport (03)-721	256	256	256	128	4	4	16	8	0.0156	0.03125	0.0156	0.0078	0.03125	0.03125	0.125	0.0625
MT 1861	S. Choleraesuis χ3246	4	4	2	2	4	8	8	16	0.0156	0.03125	0.0156	0.0078	0.0625	0.0625	0.125	0.125
	Clinical Breakpoints:	S ≤ 16, R ≥ 32[13]				S ≤ 16, R ≥ 32				S ≤ 1, I = 2, R ≥ 4				S ≤ 0.5; I = 1; R ≥ 2[12]			
MT 3277	Seattle 1946; O6 biotype 1 ATCC 25922	8	8	2	8	2	4	16	4	0.0078	0.0078	0.0078	0.0039	0.0156	0.0156	0.0625	0.0156
MT 3266	UPEC J96	8	8	4	8	128	256	1024	512	0.125	0.125	0.0625	0.0625	0.25	0.25	1	0.5
MT 2478	UPEC ECR12	8	8	4	8	64	128	512	512	0.125	0.25	0.125	0.0625	0.125	0.25	1	0.5
MT 1941	UPEC ATCC 11775	8	8	2	8	2	4	16	8	0.0156	0.0156	0.0156	0.0078	0.0156	0.03125	0.125	0.0156
MT 1864	APEC χ7126	4	4	2	4	2	2	8	4	0.0078	0.0078	0.0078	0.0078	0.0078	0.0156	0.0625	0.0078
MT 1863	A96 χ7117	4	4	2	4	2	2	8	4	0.0078	0.0078	0.0078	0.0039	0.0156	0.0156	0.0625	0.0156
MT 1860	EPEC χ2927	8	8	4	8	256	256	1024	2048	0.25	0.25	0.25	0.25	0.25	0.5	1	1
MT 1859	RDEC-1 χ2862	8	8	4	8	4	8	16	16	0.0156	0.0156	0.0156	0.0078	0.0156	0.03125	0.0625	0.03125
MT 1854	EPEC JPN 15	8	8	4	8	256	512	2048	2048	0.125	0.25	0.125	0.125	0.25	0.25	1	0.5
	Clinical Breakpoints:					S ≤ 16, R ≥ 32				S ≤ 1, I = 2, R ≥ 4				S ≤ 0.5; I = 1; R ≥ 2[12]			
MT 2289	*Y. pseudotuberculosis* YPIII/pIB1	4	8	4	4	0.5	NA	NA	2	0.0078	0.0156	0.0039	0.0039	0.0039	NA	NA	0.0156
MT 3015	*Y. pseudotuberculosis* IP32953	4	8	8	4	1	2	8	2	0.0156	0.03125	0.0156	0.0078	0.0078	NA	NA	0.03125
MT 3018	*Y. pseudotuberculosis* IP2515	4	4	4	4	1	2	4	2	0.0156	0.0156	0.0078	0.0078	0.0078	0.0156	0.03125	0.0156
MT 3019	*Y. pseudotuberculosis* IP2666	4	8	4	4	1	2	4	2	0.0078	0.0156	0.0078	0.0078	0.0078	NA	NA	0.0156
	Clinical Breakpoints:					S ≤ 16, R ≥ 32				S ≤ 1, I = 2, R ≥ 4				S ≤ 0.5; I = 1; R ≥ 2[12]			
MT 1942	*Shigella flexneri* ATCC 29903	1	1	1	1	2	NA	NA	8	0.0156	0.0156	0.0156	0.0078	0.0156	0.03125	0.125	0.0625
MT 1944	*Providencia stuartii* ATCC 29914	8	8	4	8	4	4	8	4	0.0156	0.03125	0.0156	0.0156	0.0625	0.0625	0.125	0.125
MT 1946	*Citrobacter freundii* ATCC 8090	4	8	4	8	4	4	8	8	0.0039	0.0039	0.0078	0.0039	0.0156	0.0156	0.03125	0.03125
MT 1947	*Klebsiella pneumoniae* ATCC 13883	8	8	8	8	4	NA	NA	16	0.03125	NA	NA	0.03125	0.03125	0.0625	0.125	0.125
	Clinical Breakpoints:									S ≤ 1, I = 2, R ≥ 4							
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	512	512	256	256	256	128	512	256	0.125	0.125	0.25	0.25	1	1	4	2

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

Values marked with "NA" were disregarded due to an effect of Tris buffer on antimicrobial susceptibility.

[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated

[b] Previously published values, obtained in a separate experiment

a)

		Cephalothin						Ceftiofur						Ceftriaxone					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
MT3322	MRSA USA300	32	8	16	8	8	8	64	8	8	4	2	0	512	32	32	64	32	32
MT3302	MRSA Blood	8	4	8	4	4	2	16	4	2	2	0	2	128	8	16	16	16	8
MT3315	MRSA Wound	8	16	16	16	16	8	16	4	2	2	0	2	64	4	8	8	8	4
MT3305	MSSA Blood	0.5	2	2	2	2	0	1	2	4	4	8	8	4	0	0	2	2	2
MT3307	MSSA Wound	0.125	2	0	0	2	2	1	0	2	4	8	16	4	2	2	2	2	2
MT3309	MSSA Urine	0.25	2	2	2	0	0	1	0	2	4	8	8	4	2	2	2	2	2
MT3314	MSSA Sputum	0.25	2	2	2	0	0	1	0	2	4	8	8	4	2	2	2	2	2
MT3317	CoN S. lugdunensis	0.5	0	0	0	0	0	0.5	4	4	8	8	16	2	2	2	2	2	2
MT3320	CoN S. epidermidis	0.125	2	2	2	2	2	0.5	0	4	8	8	16	2	0	0	0	0	0
MT3321	CoN S. warneri	0.125	2	2	2	2	2	0.25	2	4	8	8	16	2	2	2	2	2	2

		Oxacillin						Streptomycin						Tetracycline					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
MT3322	MRSA USA300	64	4	4	4	4	2	16	4	8	16	16	16	0.5	8	4	4	4	4
MT3302	MRSA Blood	64	16	16	16	16	4	8	4	8	8	8	8	0.5	8	4	4	4	4
MT3315	MRSA Wound	32	32	32	32	8	0	8	8	8	8	8	8	0.5	4	2	2	4	2
MT3305	MSSA Blood	0.25	0	0	0	0	2	16	8	8	16	16	8	0.5	4	4	4	4	4
MT3307	MSSA Wound	0.25	2	0	0	0	2	16	4	8	8	16	16	0.5	8	8	8	8	32
MT3309	MSSA Urine	0.25	0	0	0	0	0	32	8	32	32	32	32	4	4	4	4	4	2
MT3314	MSSA Sputum	0.25	2	2	2	0	0	32	16	16	32	32	16	0.5	8	4	4	8	8
MT3317	CoN S. lugdunensis	0.5	0	0	0	0	0	4	4	8	8	8	8	0.25	4	4	4	4	4
MT3320	CoN S. epidermidis	0.125	2	2	2	2	4	4	8	8	8	4	4	0.5	8	4	4	4	8
MT3321	CoN S. warneri	0.125	0	0	0	0	0	4	4	4	8	4	8	0.5	8	16	8	4	8

		Azithromycin						Erythromycin						Chloramphenicol					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
MT3322	MRSA USA300	128	16	16	16	16	8	64	8	8	8	8	4	8	0	0	0	0	2
MT3302	MRSA Blood	128	16	16	16	16	8	128	16	16	16	16	8	8	0	0	0	0	0
MT3315	MRSA Wound	1	8	8	8	8	4	0.25	2	2	2	2	0	8	0	0	0	2	2
MT3305	MSSA Blood	>512	R	R	R	R	R	>512	R	R	R	R	R	8	0	0	0	0	2
MT3307	MSSA Wound	2	16	16	16	16	8	1	8	8	8	8	4	8	2	2	2	2	4
MT3309	MSSA Urine	1	8	8	8	8	4	0.5	4	4	4	2	2	8	0	0	0	2	2
MT3314	MSSA Sputum	2	16	16	16	16	4	1	8	8	8	8	2	8	0	0	0	2	4
MT3317	CoN S. lugdunensis	0.25	4	4	4	4	2	0.125	2	2	2	2	0	4	2	2	2	2	2
MT3320	CoN S. epidermidis	256	8	8	8	16	8	128	4	8	8	4	4	8	0	2	2	2	2
MT3321	CoN S. warneri	256	8	8	8	8	4	256	8	8	8	8	8	8	2	2	2	2	4

[a] Previously published values, obtained in a separate experiment

[b] Previously published values, obtained again in this experiment

8+	≥8-fold resistant
4	4-fold resistant
2	2-fold resistant
=	no change
2	2-fold susceptible
4	4-fold susceptible
8+	≥8-fold susceptible
R	Resistant

0% FBS = DMEM + 5% LB
5% FBS = DMEM + 5% LB + 5% FBS
10% FBS = DMEM + 5% LB + 10% FBS
20% FBS = DMEM + 5% LB + 20% FBS
50% FBS = DMEM + 5% LB + 50% FBS

b)

		Polymyxin B						Colistin Sulfate						Ceftiofur					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
CDC 14028	S. Typhimurium 14028	0.25	8	2	8	8	8	0.5	8	4	4	4	4	0.5	0	2	4	4	2
MT 2353	S. Typhimurium TY1212	0.25	8	2	8	8	8	0.5	4	4	4	4	4	1	0	4	4	4	4
MT 1864	APEC χ7126	0.125	16	8	8	8	8	0.5	2	2	2	2	2	0.25	2	2	2	2	2
MT 1863	A96 χ7117	0.125	16	8	8	16	16	0.5	4	2	2	2	4	0.25	2	2	4	4	2
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1	8	8	8	8	16	1	16	16	16	16	32	32	0	0	0	0	2

		Ampicillin						Spectinomycin						Tetracycline					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
CDC 14028	S. Typhimurium 14028	2	0	2	0	0	2	64	2	2	2	0	0	1	8	8	8	8	8
MT 2353	S. Typhimurium TY1212	4	0	2	0	0	0	>1024	R	R	R	R	R	256	2	R	R	R	R
MT 1864	APEC χ7126	2	0	2	2	2	2	16	0	2	0	0	2	0.5	16	8	8	8	8
MT 1863	A96 χ7117	4	0	0	0	0	0	32	2	0	0	0	0	1	8	4	8	8	8
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	128	2	2	0	2	2	>512	R	R	R	R	R	64	R	R	R	R	R

		Azithromycin						Erythromycin						Chloramphenicol					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
CDC 14028	S. Typhimurium 14028	8	4	4	4	2	2	128	4	2	2	0	0	8	2	2	0	0	0
MT 2353	S. Typhimurium TY1212	8	4	4	4	2	2	128	2	2	2	0	0	512	2	2	2	2	2
MT 1864	APEC χ7126	4	2	2	2	2	0	32	2	0	2	2	2	4	2	2	2	4	4
MT 1863	A96 χ7117	4	2	2	0	0	0	64	0	2	0	0	0	8	0	0	0	2	0
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	256	8	4	8	4	2	512	4	2	2	4	2	256	2	0	0	2	2

		Nalidixic Acid						Enrofloxacin					
		Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS	Ca-MHB[a]	0% FBS[b]	5% FBS	10% FBS	20% FBS	50% FBS
CDC 14028	S. Typhimurium 14028	4	2	4	4	4	8	0.03125	4	4	4	4	4
MT 2353	S. Typhimurium TY1212	4	4	4	4	8	8	0.0625	4	4	4	4	4
MT 1864	APEC χ7126	2	2	4	4	8	8	0.0078	4	8	8	8	8
MT 1863	A96 χ7117	2	4	4	4	8	8	0.0156	4	4	4	4	4
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	256	2	2	0	2	2	1	4	4	4	4	4

[a] Previously published values, obtained in a separate experiment

[b] Previously published values, obtained again in this experiment

8+	≥8-fold resistant
4	4-fold resistant
2	2-fold resistant
=	no change
2	2-fold susceptible
4	4-fold susceptible
8+	≥8-fold susceptible
R	Resistant

0% FBS = DMEM
5% FBS = DMEM + 5% FBS
10% FBS = DMEM + 10% FBS
20% FBS = DMEM + 20% FBS
50% FBS = DMEM + 50% FBS

Figure 6. Fold change comparison of MIC values in DMEM supplemented with increasing concentrations of fetal bovine serum (FBS), relative to standard MHB. A panel of antibiotics was screened for changes in MIC values for (a) *Staphylococcus* spp. and (b) Gram-negative bacteria. Ca-MHB MIC values are shown. FBS values depict the fold-change in MICs when AST was performed in DMEM supplemented with 0%, 5%, 10%, 20%, and 50% FBS, relative to standard MHB. MIC values were obtained from at least 6 independent determinations.

Table 6a. Antimicrobial susceptibility test in media supplemented with FBS (*Staphylococcus*)

"Susceptible" MIC
"Intermediate" MIC
"Resistant" MIC

Clinical Breakpoints[a]:		Cephalothin (µg/mL) S ≤ 8; I = 16; R ≥ 32[2]						Ceftiofur (µg/mL) S ≤ 2; I = 4; R ≥ 8[3]						Ceftriaxone (µg/mL) S ≤ 8; I = 16 - 32; R ≥ 64[2]					
Strain #	Strain Name	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS
MT 3322	MRSA USA300	32	4	2	4	4	4	64	8	8	16	32	64	512	16	16	8	16	16
MT 3302	MRSA Blood	8	2	1	2	2	4	16	4	8	8	16	32	128	16	8	8	8	16
MT 3315	MRSA Wound	8	0.5	0.5	0.5	0.5	1	16	4	8	8	16	32	64	16	8	8	8	16
MT 3305	MSSA Blood	0.5	0.25	0.25	0.25	0.25	0.5	1	2	4	4	8	8	4	4	4	2	2	2
MT 3307	MSSA Wound	0.125	0.0625	0.125	0.125	0.25	0.25	1	1	2	4	8	16	4	2	2	2	2	2
MT 3309	MSSA Urine	0.25	0.125	0.125	0.125	0.25	0.25	1	1	2	4	8	8	4	2	2	2	2	2
MT 3314	MSSA Sputum	0.25	0.125	0.125	0.125	0.25	0.25	1	1	2	4	8	8	4	2	2	2	2	2
MT 3317	CoN S. lugdunensis	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2	2	4	4	8	2	4	4	4	4	4
MT 3320	CoN S. epidermidis	0.125	0.25	0.25	0.25	0.25	0.25	0.5	0.5	2	4	4	8	2	2	2	2	2	2
MT 3321	CoN S. warneri	0.125	0.0625	0.0625	0.0625	0.0625	0.0625	0.25	0.5	1	2	2	4	2	1	1	1	1	1

Clinical Breakpoints:		Oxacillin (µg/mL) S ≤ 2; R ≥ 4						Streptomycin (µg/mL) S ≤ 8; I = 16; R ≥ 32[4]						Tetracycline (µg/mL) S ≤ 4; I = 8; R ≥ 16					
Strain #	Strain Name	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS
MT 3322	MRSA USA300	64	16	16	16	16	32	16	4	2	1	1	1	0.5	4	2	2	2	2
MT 3302	MRSA Blood	64	4	4	4	4	16	8	2	1	1	1	1	0.5	4	2	2	2	2
MT 3315	MRSA Wound	32	1	1	1	4	32	8	1	1	1	1	1	0.5	2	1	1	2	1
MT 3305	MSSA Blood	0.25	0.25	0.25	0.25	0.25	0.5	16	2	2	1	1	2	0.5	2	2	2	2	2
MT 3307	MSSA Wound	0.25	0.125	0.25	0.25	0.25	0.5	16	4	2	2	1	1	0.5	4	4	4	4	16
MT 3309	MSSA Urine	0.25	0.25	0.25	0.25	0.25	0.25	32	4	1	1	1	1	4	16	16	16	16	8
MT 3314	MSSA Sputum	0.25	0.125	0.125	0.125	0.25	0.25	32	2	2	1	1	2	0.5	4	2	2	4	4
MT 3317	CoN S. lugdunensis	0.5	0.5	0.5	0.5	0.5	0.5	4	1	0.5	0.5	0.5	0.5	0.25	1	1	1	1	1
Clinical Breakpoints:		S ≤ 0.25; R ≥ 0.5																	
MT 3320	CoN S. epidermidis	0.125	0.25	0.25	0.25	0.25	0.5	4	0.5	0.5	0.5	1	1	0.5	4	2	2	2	4
MT 3321	CoN S. warneri	0.125	0.125	0.125	0.125	0.125	0.125	4	1	1	0.5	1	0.5	0.5	4	8	4	2	4

Clinical Breakpoints:		Azithromycin (µg/mL) S ≤ 2; I = 4; R ≥ 8						Erythromycin (µg/mL) S ≤ 0.5; I = 1 - 4; R ≥ 8						Chloramphenicol (µg/mL) S ≤ 8; I = 16; R ≥ 32					
Strain #	Strain Name	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS	Ca-MHB [b]	DMEM + 5% LB	DMEM + 5% LB + 5% FBS	DMEM + 5% LB + 10% FBS	DMEM + 5% LB + 20% FBS	DMEM + 5% LB + 50% FBS
MT 3322	MRSA USA300	128	8	8	8	8	16	64	8	8	8	8	16	8	8	8	8	8	16
MT 3302	MRSA Blood	128	8	8	8	8	16	128	8	8	8	8	16	8	8	8	8	8	8
MT 3315	MRSA Wound	1	0.125	0.125	0.125	0.125	0.25	0.25	0.125	0.125	0.125	0.125	0.25	8	8	8	8	16	16
MT 3305	MSSA Blood	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	8	8	8	8	16
MT 3307	MSSA Wound	2	0.125	0.125	0.125	0.125	0.25	1	0.125	0.125	0.125	0.125	0.25	8	16	16	16	16	32
MT 3309	MSSA Urine	1	0.125	0.125	0.125	0.125	0.25	0.5	0.125	0.125	0.125	0.25	0.25	8	8	8	8	16	16
MT 3314	MSSA Sputum	2	0.125	0.125	0.125	0.125	0.5	1	0.125	0.125	0.125	0.125	0.5	8	8	8	8	16	32
MT 3317	CoN S. lugdunensis	0.25	0.0625	0.0625	0.0625	0.0625	0.125	0.125	0.0625	0.0625	0.0625	0.0625	0.125	4	8	8	8	8	8
MT 3320	CoN S. epidermidis	256	32	32	32	16	32	128	32	16	16	32	32	8	8	16	16	16	16
MT 3321	CoN S. warneri	256	32	32	32	32	64	256	32	32	32	32	32	8	16	16	16	16	32

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant
Values marked with "NA" were disregarded due to an effect of Tris buffer on antimicrobial susceptibility.
[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated
[b] Previously published values, obtained in a separate experiment

Table 6b. Antimicrobial susceptibility test in media supplemented with FBS (Gram-negative bacteria)

		Polymyxin B (µg/mL)					
	Clinical Breakpoints[a]:	S ≤ 2, R ≥ 4[8]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	0.25	2	0.5	2	2	2
MT 2353	S. Typhimurium TY1212	0.5	2	0.5	2	2	2
MT 1864	APEC χ7126	0.25	2	1	1	1	1
MT 1863	A96 χ7117	0.5	2	1	1	2	2
	Clinical Breakpoints:	S ≤ 2, I = 4, R ≥ 8					
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1	8	8	8	8	16

		Colistin Sulfate (µg/mL)					
	Clinical Breakpoints:	S ≤ 2, R ≥ 4[6]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	0.25	4	2	2	2	2
MT 2353	S. Typhimurium TY1212	0.5	2	2	2	2	2
MT 1864	APEC χ7126	0.5	1	1	1	1	1
MT 1863	A96 χ7117	0.5	2	1	1	1	2
	Clinical Breakpoints:	S ≤ 2, I = 4, R ≥ 8					
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1	16	16	16	16	32

		Ceftiofur (µg/mL)					
	Clinical Breakpoints:	S ≤ 2, I = 4, R ≥ 8[9]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	1	0.5	0.25	0.125	0.125	0.25
MT 2353	S. Typhimurium TY1212	1	1	0.25	0.25	0.25	0.25
MT 1864	APEC χ7126	0.25	0.125	0.125	0.125	0.125	0.125
MT 1863	A96 χ7117	0.25	0.125	0.125	0.0625	0.0625	0.125
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	64	32	32	32	32	64

		Ampicillin (µg/mL)					
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	4	2	1	2	2	1
MT 2353	S. Typhimurium TY1212	8	4	2	4	4	4
MT 1864	APEC χ7126	4	2	4	4	4	4
MT 1863	A96 χ7117	8	4	4	4	4	4
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	256	256	256	128	256	256

		Spectinomycin (µg/mL)					
	Clinical Breakpoints:	S ≤ 32; I = 64-128; R ≥ 256[10]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	64	32	32	32	64	64
MT 2353	S. Typhimurium TY1212	>1024	>1024	>1024	>1024	>1024	>1024
MT 1864	APEC χ7126	16	16	32	16	16	32
MT 1863	A96 χ7117	16	16	32	32	32	32
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1024	512	512	512	1024	1024

		Tetracycline (µg/mL)					
	Clinical Breakpoints:	S ≤ 4, I = 8, R ≥ 16					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	2	8	8	8	8	8
MT 2353	S. Typhimurium TY1212	256	128	>512	>512	>512	>512
MT 1864	APEC χ7126	1	8	4	4	4	4
MT 1863	A96 χ7117	1	8	4	8	8	8
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	64	>512	>512	>512	>512	>512

		Azithromycin (µg/mL)					
	Clinical Breakpoints:	S ≤ 16, R ≥ 32[6]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	8	2	2	2	4	4
MT 2353	S. Typhimurium TY1212	8	2	2	2	4	4
MT 1864	APEC χ7126	4	2	2	2	2	4
MT 1863	A96 χ7117	8	2	2	4	4	4
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	256	32	64	32	64	128

		Erythromycin (µg/mL)					
	Clinical Breakpoints:						
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	128	32	64	64	128	128
MT 2353	S. Typhimurium TY1212	256	64	64	64	128	128
MT 1864	APEC χ7126	64	64	32	64	64	64
MT 1863	A96 χ7117	64	64	32	64	64	64
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	512	128	256	256	128	256

		Chloramphenicol (µg/mL)					
	Clinical Breakpoints:	S ≤ 8, I = 16, R ≥ 32					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	8	4	4	8	8	8
MT 2353	S. Typhimurium TY1212	512	256	256	256	256	256
MT 1864	APEC χ7126	4	8	8	8	16	16
MT 1863	A96 χ7117	8	8	8	8	16	8
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	256	128	256	256	512	512

		Nalidixic Acid (µg/mL)					
	Clinical Breakpoints:	S ≤ 16, R ≥ 32					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	4	8	16	16	16	32
MT 2353	S. Typhimurium TY1212	4	16	16	16	32	32
MT 1864	APEC χ7126	2	4	8	8	16	16
MT 1863	A96 χ7117	2	8	8	8	16	16
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	512	512	512	256	512	512

		Enrofloxacin (µg/mL)					
	Clinical Breakpoints:	S ≤ 0.5; I = 1; R ≥ 2[12]					
Strain #	Strain Name	Ca-MHB [b]	DMEM	DMEM + 5% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 50% FBS
CDC 14028	S. Typhimurium 14028	0.0625	0.125	0.125	0.125	0.125	0.125
MT 2353	S. Typhimurium TY1212	0.0625	0.25	0.25	0.25	0.25	0.25
MT 1864	APEC χ7126	0.0156	0.03125	0.0625	0.0625	0.0625	0.0625
MT 1863	A96 χ7117	0.0156	0.0625	0.0625	0.0625	0.0625	0.0625
	Clinical Breakpoints:						
MT 1945	*Pseudomonas aeruginosa* ATCC 10145	1	4	4	4	4	4

All MICs were determined by broth microdilution in accordance with CLSI guidelines. DMEM MICs were incubated in a 5% CO2 incubator. S = Susceptible, NS = Non-Susceptible, I = Intermediate, R = Resistant

[a] All Clinical Breakpoints are referenced from the CLSI 2014 twenty-fourth informational supplement[1] unless otherwise indicated

[b] Previously published values, obtained in a separate experiment

Table 4 & Table 6 References

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Chapter 4: Conclusions and Future Directions

The antibiotic crisis is a global threat and must be made a priority for research and clinical settings. While measures such as vaccination and hygienic practices are in place to reduce the spread of infection disease, infectious disease is still among the top 10 causes of death worldwide (Centers for Disease Control and Prevention, 2011). To help resolve this ongoing problem, we investigated novel methods to improve the accuracy of antimicrobial susceptibility testing and thus encourage proper use of antimicrobials and reduce instances of antibiotic resistance.

In Chapter 2, the antimicrobial susceptibility profiles of pathogenic bacteria were investigated in various host-mimicking media to provide a more thorough understanding of the scope of altered susceptibility. We hypothesized that due to the different environmental signals in host-mimicking media compared to MHB, levels of antimicrobial resistance would significantly differ between media. Additionally, we predicted that the susceptibility profiles obtained in host-mimicking media would be more representative of an *in vivo* infection. We discovered that there were several instances of drugs showing high levels of resistance in host-mimicking media that showed susceptibility in MHB and therefore may have incorrectly been considered as a valid treatment option. Alternatively, we found that certain strains were susceptible to a particular drug in host-mimicking media but found to be resistant in MHB, implying that they were susceptible to drugs that would have otherwise been excluded as a treatment option. Importantly, many of the predicted susceptibility profiles in host-mimicking media were proven accurate in

animal testing models and therefore contradicted values obtained with the standard MHB. Also in Chapter 2 we introduced $NaHCO_3$ as a signaling molecule that influenced antimicrobial susceptibility. In several cases, the susceptibility profile obtained with host-mimicking media was observed by adding $NaHCO_3$ to MHB. Although this method could not be applied to every strain in every case, it is a helpful step in expanding the possibilities of *in vitro* testing to better predict treatment outcomes.

In Chapter 3, we completed the screening of the $NaHCO_3$ data described in Chapter 2 to better understand the scope of the effect of sodium bicarbonate on antimicrobial susceptibility. In addition, we investigated the antimicrobial susceptibility profiles that resulted from supplementing media with fetal bovine serum (FBS). We hypothesized that supplementing host-mimicking media with various levels of FBS would create susceptibility profiles that not only differed from MHB, but also would display a dose response to the levels of FBS. We discovered that supplementing host-mimicking media with FBS further led to susceptibility profiles that significantly differed from those obtained with MHB. Additionally, dose-dependent responses were observed in some cases as the susceptibility gradually increased or decreased with the change in %FBS. Overall these results demonstrated that signals in the extracellular environment clearly play a role in the bacteria's signaling process in a way can alter their susceptibility to a given drug.

Ultimately, our findings suggest that the results of AST are often dependent on the presence of certain signals found in the extracellular environment. Therefore, it should be recognized that utilizing the optimal host-mimicking media for a particular

strain is necessary to obtain accurate AST results. Since there is no single media that will provide accurate test results for all strains in every case, MIC testing should be conducted using multiple testing media containing signals that may be encountered by the given pathogenic strain in the host environment. This would provide valuable insight into the commonly contradicting MIC values obtained between host-mimicking and standard media, and this knowledge would be well worth the additional cost to routinely complete these experiments. As explained previously, incorrect prescription of drugs leads to selection of resistant bacteria and inability to improve the patient's condition. Accurate AST results and proper use of antibiotics is crucial in helping solve the antibiotic resistance crisis. Therefore, by performing AST in multiple host-mimicking media alongside MHB, we obtain more information to support the decision of which drug to treat with.

Chapter 5: Materials and Methods

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5.1 Bacterial Strains and Media

Staphylococcal clinical isolates analyzed included USA300, a community-associated methicillin-resistant *Staphylococcus aureus* (*SA*) isolate causing the most MRSA infections in the United States (Diekema et al., 2014); and 9 isolates from human sepsis patients (Santa Barbara Cottage Hospital, 2016) with various host sites of pathogen origin including blood, wound, urine, sputum (termed MRSA Blood [MT3302]; Wound [MT3315]); MSSA (Blood [MT3305]; Wound [MT3307]; Urine [MT3309]; Sputum [MT3314]); and CoNS (*S. epidermidis*, blood [MT3320]; *S. lugdunensis*, blood [MT3317]; *S. warneri*, blood [MT3321]). *S. pneumoniae* (*SPN*) clinical isolates included D39 (ser. 2) (Lanie et al., 2007), and 5 *SPN* isolates derived from the nasopharynx of children with sickle cell anemia at risk for invasive pneumococcal disease (Daw 1 [serotype 6]; Daw 2 [serotype 23]; Daw 19 [serotype 6]; Daw 20 [serotype 11]; Daw 25 [serotype 35C]) (Carter et al., 2014; Daw et al., 1997). Gram-negative bacterial isolates included *Salmonella* spp., *Salmonella* Typhimurium ATCC 14028, TY1212; and var. 5 (04)-9639; *S*. Dublin Lane; *S*. Newport (03)-721; *S*. Choleraesuis χ3236 (Heithoff et al., 2012; Heithoff et al., 2008); *E. coli* ATCC 25922; UPEC J96; UPEC ECR12; UPEC ATCC 11775; APEC χ7126; A96 χ7117; EPEC χ2927); RDEC-1 χ2862; EPEC JPN 15; *Yersinia pseudotuberculosis* (YPIII/pIB1; IP32953; IP2515; IP2666) (Kubicek-Sutherland,

Heithoff, Ersoy, Shimp, & Mahan, 2014); *Shigella flexneri* ATCC 29903; *Providencia stuartii* ATCC 29914; *Citrobacter freundii* ATCC 8090; *Klebsiella pneumoniae* ATCC 13883; *Pseudomonas aeruginosa* ATCC 10145. All *Staphylococcus* strains were isolated on Tryptic Soy Broth Agar (TSA) incubated at 37 °C in ambient air. *S. pneumoniae* strains were isolated on Columbia Sheep's Blood Agar (CSBA) and grown in Todd-Hewitt Broth (THB) supplemented with 2% yeast extract incubated at 37 °C in a 5% CO_2 incubator. Gram-negative bacteria were isolated on Luria-Bertani (LB) agar (Davis, 1980) incubated at 37 °C or 28 °C (*Yersinia*) in ambient air. Standard AST broth medium is Mueller–Hinton Broth (MHB) supplemented with CaCl2 and MgCl2 to make cation-adjusted MHB (Ca-MHB) (Clinical and Laboratory Standards Institute, 2012a). AST was also performed in Dulbecco's Modified Eagle Medium (DMEM (Dulbecco & Freeman, 1959); High Glucose [Life Technologies]); Lacks medium (Lacks, 1966); modified Lacks medium (MLM) (Hathaway et al., 2012); low phosphate, low magnesium medium (LPM) (Coombes et al., 2004); and Fetal Bovine Serum (FBS; qualified, USDA-approved regions [Life Technologies]). DMEM and FBS cultures were incubated in a 5% CO_2 incubator; all other conditions were incubated in ambient air. To facilitate growth, DMEM was supplemented with 5% LB broth for *Staphylococci*, and 5% Lysed Horse Blood (LHB) for *S. pneumoniae*; MLM was supplemented with 5% THB for *S. pneumoniae* D39.

5.2 MIC Assays

The minimum inhibitory concentration (MIC) was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines by either broth or agar dilution (Clinical and Laboratory Standards Institute, 2012a; Wiegand et al., 2008).

For determination of MIC in alternative media conditions, bacteria were obtained from overnight culture (*Staphylococci* and Gram-negative bacteria) or after a 4 h incubation period (*S. pneumoniae*) in specified medium and diluted into same medium containing 2-fold serial dilutions of antibiotics. To control for the potential effects of pH and media composition for LPM pH 5.5 comparisons, antibiotic resistance and clinical breakpoint designations were calculated by comparing the MIC in LPM medium divided by the MIC in MHB medium at both pH 5.5 and pH 7 (unbuffered) (ratio of LPM pH 5.5/pH 7.0 to MHB pH 5.5/pH 7.2) (Kubicek-Sutherland et al., 2015). MIC values were derived after 20 h incubation, and were the result of at least 6 independent determinations.

5.3 Sodium Bicarbonate Susceptibility Assays

Strains were grown in MHB pH 7.2; unbuffered; MHB adjusted to pH 7.2 with 100 mM Tris(hydroxymethyl)aminomethane (Fisher Scientific); and DMEM liquid pH 7.4 (containing 44 mM $NaHCO_3$; Difco/Becton Dickinson). All other media conditions were adjusted to pH 7.4 with 100 mM Tris including: MHB medium w/$NaHCO_3$; and $NaHCO_3$-free powdered DMEM w/wo $NaHCO_3$. Bacteria were grown overnight in specified medium and diluted as described above. For *S. pneumoniae* isolates, $NaHCO_3$ assays were performed in MHB medium in the CO_2 incubator due to viability considerations since *S. pneumoniae* isolates tested did not grow in either MHB medium with $NaHCO_3$ in ambient air; or in DMEM in the absence of $NaHCO_3$ in the CO_2 incubator. MIC values were the result of at least 6 independent determinations completed on at least two days. Control MICs in MHB or DMEM were always performed alongside the experimental media conditions.

5.4 Virulence Assays

5.4.1 Intraperitoneal (i.p.) Infection

S. Typhimurium 14028 (dose of 102 CFU) and *S. pneumoniae* Daw 25 (dose of 9 × 107 CFU) were grown overnight in LB or Todd-Hewitt medium with 2% yeast extract, respectively, and sub-cultured to A600 = 0.4, resuspended in 0.15 M NaCl, and administered to mice via the i.p. route of infection.

5.4.2 Intravenous (i.v.) Infection

MRSA USA300 (dose 1 × 108 CFU), MRSA Blood (MT3302; dose 1.5 × 108 CFU) and MSSA Wound (MT3307; dose of 2 × 108 CFU) were grown overnight in TSB and sub-cultured to A600 = 0.4; and *K. pneumoniae* ATCC 13883 (dose of 2 × 108 CFU) were grown overnight in LB medium. Strains were resuspended in 0.15 M NaCl and administered i.v. to mice by retro-orbital injection.

5.4.3 Antibiotic Treatment

Infected mice were treated (or mock-treated) with the following dosing regimens beginning 2 h post-infection: azithromycin (100 mg/kg/day), ceftiofur (40 mg/kg/day), ceftriaxone (50 mg/kg/day), cephalothin (200 mg/kg/day), ciprofloxacin (30 mg/kg/day), colistin (30 mg/kg/day), co-trimoxazole (15 mg/kg/day), daptomycin (10 mg/kg/day), erythromycin (100 mg/kg/day), tetracycline (100 mg/kg/day), or trimethoprim (30 mg/kg/day).

5.4.4 Bacterial Clearance

Mice infected with MSSA Wound (MT3307; dose of 4 × 108 CFU) were treated with azithromycin or co-trimoxazole. All drug doses were delivered once every 24 h

except cephalothin, ciprofloxacin, colistin, and co-trimoxazole, which were delivered once every 12 h; ceftriaxone and ceftiofur were given every 12 h for MRSA Blood (MT3302) experiments. All drugs were delivered by the i.p. route with the exception of cephalothin (subcutaneous). Mouse survival was assessed for 10 days post-infection. Equal numbers of male and female 10- to 12- week-old litter-mate C57BL/6J mice were used in all virulence studies. Institutional Animal Care and Use Committee of the University of California, Santa Barbara approved all mouse research protocols undertaken herein.

5.5 Statistical Analysis

Statistical significance for difference in proportions of animal survival was calculated using Chi-square (Epi Info 7, CDC). For all statistical analyses, a significance level (P) of <0.05 was considered to be statistically significant. Degrees of statistical significance are presented as ***$P < 0.001$, **$P < 0.01$, or *$P < 0.05$.

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